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Bioanalytical method validation of Venlafaxine and Desvenlafaxine in mouse plasma, brain and liver using a MEPS/HPLC assay: a path to study these drugs' intranasal administration

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Abstract

The present thesis was elaborated aiming to obtain the master's degree in pharmaceutical sciences. In here, it is described all the scientific investigation work done to reach the proposed objective, as well as all tasks and learnings that I have taken along my community and hospital pharmacy internships.

Chapter 1 is related with my scientific investigation work entitled "Bioanalytical method validation of Venlafaxine and Desvenlafaxine in mouse plasma, brain and liver using a MEPS/HPLC assay: a path to study these drugs' intranasal administration". Since major depressive disorder is one of the most prevalent psychiatric diseases, its therapeutic successful rate must be increased. Nowadays, the majority of all antidepressants used are given orally, being this route a source of therapeutic problems, mainly in what concerns with the time and dosage required to accomplish the desired effects. So, in order to improve it, intranasal administration route has been investigated, once parts of the nasal cavity directly contacts with brain tissue. Over time, and after some research, it was also understood that Venlafaxine is one of the most successful antidepressants until now, having the advantage of being metabolized to an active metabolite called Desvenlafaxine. So, in order to perform some future studies using intranasally administration of both these drugs and understand what advantages it brings, a reliable bioanalytical quantification method for venlafaxine and desvenlafaxine in mouse plasma, brain and liver matrices must be validated, in this case, using a MEPS/HPLC method.

Chapter 2 is related with community pharmacy internship, describing all the performed activities during the 3 month that I have stayed in Pharmacy Alameda. The focus in this chapter is to understand how community pharmacies in general work, taking into account not only drugs dispense and pharmaceutical services but also the best way to manage them.

Chapter 3 is about my 2 month of internship in hospital pharmacy services, describing all the duties and tasks performed in each pharmacy section. In here it is also explicit pharmacists' importance in patients' pharmaceutical therapies management as well as in controlling all the economic costs related with it.

Keywords

Venlafaxine; Desvenlafaxine; Intranasal administration; HPLC; MEPS; Pharmacy.

Resumo

A tese aqui exposta foi elaborada com o intuito de obter o grau de Mestre em Ciências Farmacêuticas. Seguidamente é descrito todo o trabalho efetuado no âmbito da investigação científica, de modo a que o objectivo proposto fosse alcançado. Estão também descritas as tarefas elaboradas e aprendizagens obtidas ao longo dos meus estágios em farmácia comunitária e em farmácia hospitalar.

No Capítulo 1 é descrito todo o meu trabalho no âmbito da área de investigação, sendo este intitulado "Validação de um método bioanalítico de Venlafaxina e Desvenlafaxina em plasma, cérebro e fígado de murganho usando MEPS/HPLC: um caminho para o estudo da administração intranasal destes fármacos". Uma vez que a depressão major é uma das doenças psiquiátricas mais prevalente, a taxa de sucesso do seu tratamento terá obrigatoriamente de ser aumentada. Actualmente, a maioria dos antidepressivos são administrados por via oral, o que é uma fonte de problemas a nível terapêutico, considerando principalmente o tempo e as elevadas doses necessárias para o tratamento da doença. Assim, de modo a melhorar este aspecto, a via intranasal tem vindo a ser estudada, uma vez que existem locais na cavidade nasal que contactam directamente com o tecido cerebral. Ao longo do tempo também se tem percebido que a Venlafaxina é um dos antidepressivos mais eficazes, tendo também a vantagem de ser metabolizada num metabolito activo chamado Desvenlafaxina. Assim, de modo a perceber quais as vantagens da administração por via intranasal da Venlafaxina e da Desvenlafaxina, deve ser antes validado um método bioanalítico usando MEPS/HPLC de modo a que ambos se consigam quantificar em plasma, cérebro e fígado de murganho.

O Capítulo 2 está relacionado com o estágio em farmácia comunitária, no qual são descritas todas as actividades realizadas ao longo dos 3 meses que passei na Farmácia da Alameda. Neste capítulo é focado todo o funcionamento em geral de uma farmácia comunitária, tendo sempre em conta não apenas a dispensa de medicamentos e os serviços farmacêuticos, mas também as melhores formas de gestão efetuadas neste âmbito.

O Capítulo 3 refere-se aos 2 meses de estágio em farmácia hospitalar, descrevendo-se aqui todas as tarefas e responsabilidades de cada secção da farmácia. Aqui é também focada a importância do farmacêutico hospitalar na gestão das terapêuticas dos doentes e dos custos associados a estas.

Palavras-chave

Venlafaxina; Desvenlafaxina; Administração intranasal; HPLC; MEPS; Farmácia

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MDD	Major depressive disorder
ADME	Absorption, distribution, metabolism and excretion
AIM	Pharmaceutical products' holders
API	Active pharmaceutical ingredient
AUC	Area under the curve
AUE	Exceptional authorization use
BBB	Blood brain barrier
BCF	Blood-cerebrospinal fluid
BMI	Body mass index
BP	Blood pressure
BZD	Benzodiazepines
CARAT	Control of allergic rhinitis and asthma test
CFT	Therapeutic and pharmacy commission
CHCB	*Centro Hospitalar Cova da Beira, E.P.E.*
C_{max}	Peak serum concentration
CNP	Product national code
CNPEM	*Código nacional de prescrição eletrónica de medicamentos*
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CSF	Cerebrospinal fluid
CV	Coefficient of variation
CYP	Cytochrome P450
EMs	Extensive metabolizers
FDS	Fast dispensing system
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GIT	Gastrointestinal tract
GOLD	Global initiative for chronic obstructive lung disease
HL	Hospital logistic
HPLC	High-performance liquid chromatography
ICU	Intensive care unit
IDDSD	Individual dairy distribution in daily dosage
IN	Intranasal

INFARMED	*Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.*
INM	International non-property name
IS	Internal standard
IV	Intravenous
IVA	*Imposto de venda ao público*
JCI	Joint Commission International
LASA	Look-alike-sound-alike
LIC	Licarbazepine
LLOQ	Lower limit of quantification
MAO	Monoamine oxidase
MDR	Multidrug resistant protein
MNSRM-DEF	Non-prescription drugs exclusively dispensed in pharmacies
MEPS	Microextraction by package sorbent
mMRC	Modified medical research counseling dyspnea scale
NHMF	National hospital medication formulary
OA	Operational assistant
ODV	Desvenlafaxine/*O-desmethylvenlafaxine*
OTCs	Over-the-counter
PA	Pharmacy Alameda
PDA	Personal digital assistant
P-gp	P-glycoprotein
PL	Portuguese legislation
PMs	Poor metabolizers
PS	Pharmaceutical services
PVA	Prices in reference countries
PVP	Public selling prices
QC	Quality control
QC_1	Low quality control
QC_2	Medium quality control
QC_3	High quality control
r^2	Coefficient of determination
RE	*Receita especial*
SAM	Medical support system
SGICM	Drugs circuit integrated management system
SNRIs	Serotonin-norepinephrine reuptake inhibitors

SNS	Health National System
SSRIs	Selective serotonin reuptake inhibitors
$t_{1/2}$	Half-life
TCAs	Antidepressants
TCA	Trichloroacetic acid
TD	Technical director
TDT	Therapeutic and diagnose technician
T_{max}	Time to peak concentration
UCAD	*Unidade de cuidados agudos e continuados*
UMs	Ultra-rapid metabolizers
VEN	Venlafaxine

Chapter 1 – Bioanalytical method validation of Venlafaxine and Desvenlafaxine in mouse plasma, brain and liver using a MEPS/HPLC assay: a path to study these drugs' intranasal administration

1.1 Introduction

Major depressive disorder (MDD) is one of the most prevalent and debilitating psychiatric disorders. It is mainly characterized by encompassing low mood, low self-esteem and loss of interest and pleasure in normal daily activities [1]. According to Diagnostic and Statistical Manual of Mental Disorders (DSM-V), American Psychiatric Association (2015), the criteria to consider a major depressive episode are the presence of, at least, five of the following symptoms during a period of two consecutive weeks, in which it is mandatory that one of them is depressive humor during the day, almost all days (children and teenagers can present irritating mood), or loss of pleasure or interest in daily activities, almost all days. The others could be: weight and/or appetite gain/loss, insomnia or hypersomnia, psychomotor agitation or slowness almost all days, fatigue and loss of energy, feelings of excessive guilt and depreciation, decrease of concentration and thought capacity, and finally recurrent dead thoughts, mainly related to suicide and without a preliminary, concrete plan to do it. All this aspects need to occur almost all days during the previous cited period of time, and cannot be associated with some other physiologic disease, substance abuse or a life landmark/traumatic event. They also need to affect the social, occupational or another area of the patient life [2].

MDD affects around 121 million of people worldwide and is responsible of nearly 850.000 deaths every year. Some researches show that 15% of the population from high-income countries, compared to 11% for low/middle-income countries, was likely to get depression over their lifetime, being twice more common in women than in men across all ages groups, with the exception of 18-34 age group, in which the classical female:male odds ratio of MDD is reduced [3,4].

Throughout years, and even more in the last decade, the prescription of antidepressant drugs has increased [5] and the discovery of tricyclic compounds and monoamine oxidase inhibitors, effective in treatment of MDD, lead to several hypotheses for pathophysiological mechanisms that causes depression, being the monoamine hypothesis the most widely accepted. This one is based on facts that brain monoaminergic (serotonin, norepinephrine and dopamine) neurotransmission is affected. So, these neurotransmitters are the critical targets to all antidepressants and, if they are synergistically targeted, the effect will be even more significant [6,7]. The majority of drugs intended to treat MDD enhance monoamines availability

in the synapse, even by inhibiting their intraneuronal metabolism, increasing their release by blocking the α_2 auto and hetero-receptors and, mainly, through inhibition of their neuronal reuptake in the presynaptic neurons [6].

Unfortunately, most of the MDD cases are therapeutically refractory or resistant to medication. This is caused by several factors including not only patients personal characteristics but also socioeconomic conditions, demography, educational level, environmental factors (nutrition, work hours, stress), adherence to the long term therapy and so on. Polygenetic variations, physiological and pathophysiological factors (age, gender, pregnancy and concomitant diseases, mostly hepatic and renal ones) are other important aspects to consider [8]. An important point related with antidepressant resistance is the limitations that drugs faced to reach the brain, especially if they are taken orally or systemically (some of the problems described below do not apply to systemic route of administration, such as first-pass metabolism). The causes are multiple but are mainly related with transport restrains from systemic circulation to central nervous system (CNS). These restrictions are related with some barriers like the blood brain barrier (BBB), blood-cerebrospinal fluid (BCF) barrier and others. Brain capillary vessels and choroid plexus are in their nature, being it the major impediments for molecules to reach the target receptors in brain (biophase) [5,10], especially for charged molecules with a high molecular weight. However, once they keep away foreign materials from brain, it can be an advantage in case of toxic and harmful particles entrance in the body [11]. Some other important limitations need to be considered in the variability of clinical antidepressant responses. First-pass metabolism and other metabolic pathways, genetic polymorphisms, plasma protein binding levels that affects both action duration, intensity and drugs BBB passage [11], efflux proteins [e.g. P-glycoprotein – (P-gp)] present in several important path organs, and drug interactions due to co-medication are the main concerns. This last aspect has been greatly considered in the last years because of the world's population aging, the increase of other psychiatric, neurologic and somatic diseases that came with MDD, and the daily hazardous exposures that lead to the development of other acute or chronic diseases. Consequently, polypharmacy and the requirement of extended periods for MDD treatment carry an increased risk of drug-drug interactions [e.g. inhibition/induction of P-gp and/or metabolic cytochrome P450 (CYP) isoenzymes] [12-14], which can modify the pharmacokinetics of antidepressant drugs even in a way that can lead to severe adverse effects and poor tolerability and efficacy. This is more relevant especially in the elderly, an age group where depression levels are increasing and that usually are already under complex polytherapy regimens. So, in this age group, the refractory depression occurrence probability is even higher, leading to the need of adding other antidepressants to improve clinical response [8].

1.1.1 Venlafaxine Pharmacology: pharmacokinetic and pharmacodynamics

Nowadays, venlafaxine (VEN) is one of the most commonly prescribed antidepressants worldwide, mainly in second line therapy, being used as an alternative drug in the treatment of selective serotonin reuptake inhibitors (SSRIs) resistant depression. That is because it is a good choice for non-remitting patients due to its particular pharmacokinetics profile, which gives it a favorable tolerability when compared to others.

It was first synthesized by Wyeth in 1993 and it was the first one of this antidepressant group that aimed to treat both MDD and anxiety disorders. Chemically, it is a hydroxycycloalkylphenylethylamino (Figure 1) derivative bicyclic antidepressant, belonging to the class of serotonin-norepinephrine reuptake inhibitors (SNRIs) [8,12,15]. Its mechanism of action is based on a dual action in serotonin and norepinephrine reuptake inhibition, by blocking receptors in the pre-synaptic neurons and other paths responsible of capturing these neurotransmitters. Consequently, it will increase the diminished levels in the synaptic cleft and the response of post synaptic neurons to them. What also contributes to the efficacy of VEN is its uninterrupted presence at the site of action over a sustained period of time [1]. It is used as a racemic mixture of the R-(-)-enantiomer and S-(+)-enantiomer, in which the first one inhibits both serotonin and norepinephrine reuptake, and the second one only inhibits serotonin reuptake [5]. Although the two have similar absorption and distribution properties, the dual inhibitory effect of R-(-)-enantiomer only became meaningful at higher doses (150mg/day or more), so, generally, VEN is more potent in blocking serotonin uptake than that for norepinephrine [7,8]. Studies in rats have also shown that high doses, but not low ones, induced a functional desensitization of the terminal 5-HT_{1B} auto-receptors at pre-synaptic neurons and, additionally enhance tonic activation of post-synaptic 5-HT_{1A} receptors. This desensitization of the auto-receptors leads to an increase of serotonin in the synaptic cleft as a consequence of the increased reuptake inhibition. So, in short time and with a low VEN dosage, the firing activity of serotonin in post-synaptic neurons was markedly reduced because of the lower degree of desensitization of pre-synaptic auto-receptor, however, at high doses and in a long term treatment, a full recovery of the firing activity of serotonin post-synaptic receptors happened, what proves the adaptive mechanism of these neurons (this was not observed with norepinephrine neurons) [7].

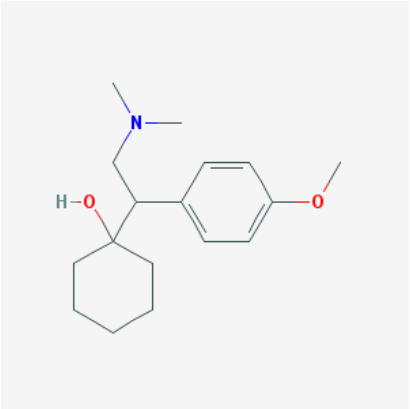

Figure 2 - Chemical representation of venlafaxine [15].

It is important to know that VEN also has some effects in inhibiting dopamine reuptake but does not have this inhibitory property in the monoamine oxidase (MAO) enzymes family, which is responsible for catalyzing the oxidation of monoamines like serotonin,

norepinephrine, dopamine, and other neurotransmitters. VEN also has no significant affinity for α_2-adrenergic, muscarinic, cholinergic, H_1-histaminergic, benzodiazepine (BZD) and opioid receptors [13,14]. So, contrary to SSRIs and tricyclic antidepressants (TCAs), VEN has a low potential to cause anticholinergic and orthostatic hypotensive effects, as well as sedation and weight gain. However, it is particularly important the blood pressure increase caused by VEN, mostly for patients that already suffer of arterial hypertension disease [14].

Another shortcomings mainly related to oral therapy are the slow onset of action, systemic side effects like tachycardia, fatigue, headache, dizziness, sexual dysfunction, dry mouth and the need of frequent oral administrations to maintain an effective therapeutic concentration in blood [1]. This last point forwards us to the pharmacokinetic properties of VEN, which are the justification for it. It is well absorbed from gastrointestinal tract (GIT) after oral administration (~92%), but its bioavailability is only of 40-45%. This is due to its extensive first-pass metabolism, responsible for its major metabolite in serum *O-desmethylvenlafaxine* (ODV) synthesis, most commonly known by desvenlafaxine. This one has an equivalent pharmacological activity and potency compared with the parent drug, so the therapeutic consequences of this conversion aren't meaningful. Besides it, other minor metabolites, with few or no relevant pharmacological activity at all, are formed in parallel, such as *N,O-didesmethylvenlafaxine, N-desmethylvenlafaxine* and *N,N,O-tridesmethylvenlafaxine* (Figure 2) [5,8,12,16]. This biotransformation is catalyzed by the CYP isoenzymes, in which ODV formation happens due to CYP2D6, especially for VEN R-enantiomer. Consequently, the plasma concentration of this active metabolite is two- to three-times fold higher than VEN. The other isoenzymes responsible for minor metabolites formation, through demethylation of the parent drug and metabolites already formed, are mainly CYP3A4, CYP2C19 and CYP1A2. Then, after these oxidative pathways, they are conjugated in phase II metabolic biotransformation lanes, forming *aryl-O-glucuronide* metabolites that are then excreted due to an increase in their hydrophilic properties [8,16].

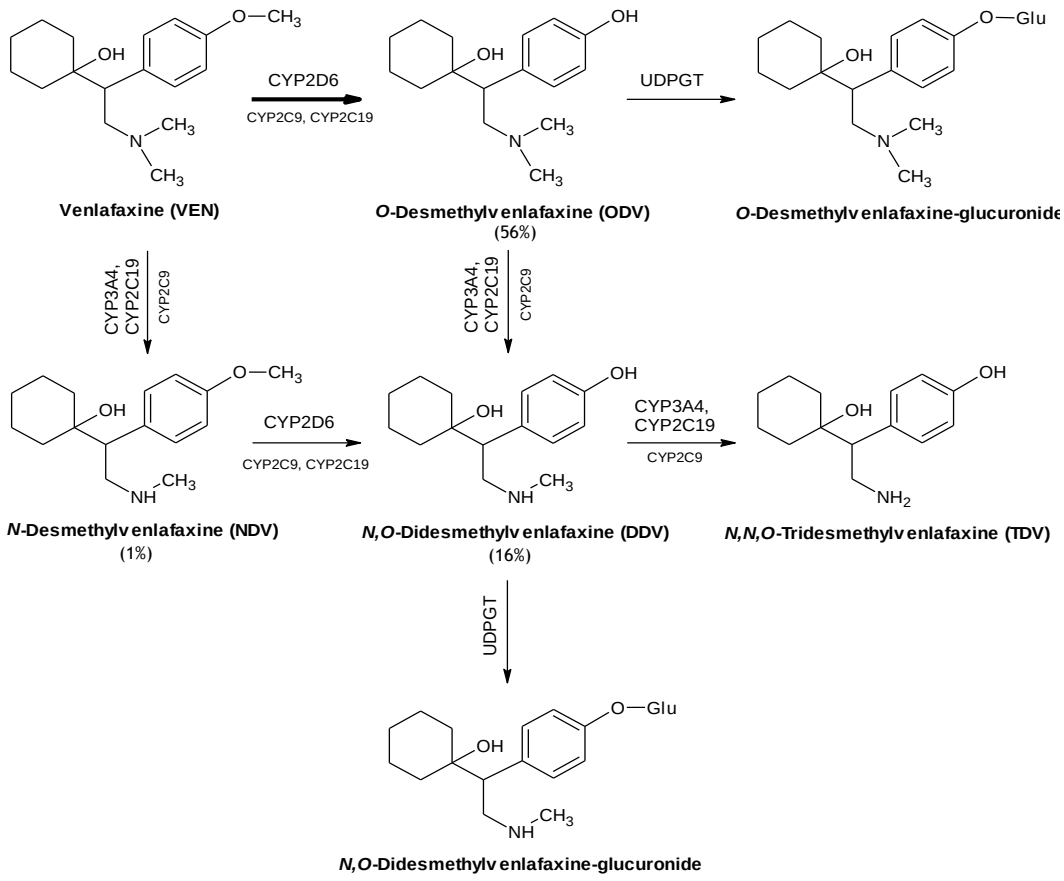

Figure 2 - Venlafaxine metabolic pathways and correspondent CYPs that catalyze each step of it. Note that the major pathway catalyzed by CYP2D6 is represented with a bold arrow [8].

Once in the systemic circulation, both VEN and ODV are widely distributed throughout the body since their binding to plasma proteins is not very significant (27% for VEN and 30% for ODV). This aspect contributes to a larger water volume distribution and, consequently, to their pass through the placenta and to the breast milk. So, in post-partum women, extra careful must be had, especially in what the babies' health is concerned. Related with excretion pathways, it is described that VEN and its related compounds are primarily excreted in urine (~92.1%) and, in a few degree, in feces. Overall, the terminal elimination half-life of VEN is approximately 5±2h and for ODV is about 11±2h. An advantage of these two molecules is that they exhibit a linear relationship between dose and plasma concentrations, within a daily dose range of 75-450mg [8,17]. In order to determine both VEN and ODV peak serum concentration (C_{max}) and time to peak concentration (T_{max}), a study in humans was made using 150mg/day of an extended release formulation of VEN (Effexor XR) and 75mg of an immediate release formulation administrated at each 12 hours of VEN too (Effexor Hydrochloride) [17]. The results are shown in Table 1.

Table 4 - Cmax and Tmax of venlafaxine (VEN) and its major metabolite desvenlafaxine (ODV) studied in humans after oral administration of both extended and immediate release Venlafaxine formulations [17].

	$C_{max.}$(ng/mL)		$T_{max.}$(h)	
	VEN	**ODV**	**VEN**	**ODV**
VEN extended release 150mg/day	150ng/mL	260ng/mL	5,5h	9h
VEN immediate release 75mg/12h	225ng/mL	290ng/mL	2h	3h

In this study, it was also realized that if it is used equal doses of either immediate release tablet or an extended release capsule, the exposure of VEN and ODV are similar for both, only with a slightly lower plasma concentration fluctuation for the extended release formulation case. So, it was proved that this one provides a slower rate of absorption but the same extent of absorption, when compared with the immediate release formulation [17].

Applying what was said before about all the antidepressants in general, patients who are treated with VEN also have exhibited large inter-individual variability in their drug outcome. This can possibly be linked with the pharmacokinetics and/or pharmacodynamics influence related with genetic and non-genetic individual factors. The expression of these variations in genotype and phenotype is more evident and studied to metabolic profiles of CYP450 isoenzymes and drug transporters. This leads to a drug disposition and, later, to antidepressant response variability, since the bioavailability/biodisposition at the target receptors will be compromised according with these variations [8,9].

Talking about CYP2D6, the most important isoenzyme for VEN metabolism, there are more than 70 different alleles of its coding gene, and they all occur in variable frequencies in different ethnicities. They have been linked to three classes of phenotypes, based on drug metabolism extend: extensive metabolizers (EMs), the most frequent type in world population, poor metabolizers (PMs) and ultra-rapid metabolizers (UMs). So, as VEN and ODV concentrations are mostly dependent of CYP2D6 metabolizer state and considering ODV/VEN ratio a measurement of this CYP activity in depressed patients, it was suggested that the clinical responses and side effects of VEN are related to CYP2D6 genotype. That is why there are evidences that PMs have an increased risk of developing side effects, being the most common nausea, vomiting and diarrhea, and also present a less favorable clinical response. If we compare VEN and ODV, there are slight differences in reuptake inhibition of norepinephrine and dopamine that explain this higher frequency in side effects, being more recurrent with the parent molecule [8,9,12]. Besides it, studies reveled that CYP2D6 displays a marked stereoselectivity towards R-enantiomer and, since it has the dual effects described

earlier in neurotransmitters reuptake inhibition, this could lead to clinical consequences and cause the observed differences of occurrence of side effects in PMs and EMs [12].

Also related with these different metabolizer types, the pharmacokinetics of VEN and ODV can present some differences. According to Preskorn et al. (2009) study, after administrating an extended release formulation of VEN, its C_{max} and area under the curve (AUC) in plasma were significantly higher, by approximately 149% and 331%, respectively, in PM's compared with EM's. Also VEN half-life ($t_{1/2}$) was prolonged in PM's (12.7h) compared with EM's. Now, related to ODV pharmacokinetic parameters also determined in this study, after administration of the same previous VEN formulation, C_{max} and AUC were significantly lower by approximately 78% and 73%, respectively, for PMs compared with EMs. But, if it is administrated directly ODV instead of VEN, the mean C_{max} and AUC for ODV were statistically comparable between EMs and PMs [18]. All data are summarized in Table 2.

Table 5 - Summary of the effect of metabolizers' status on venlafaxine and desvenlafaxine pharmacokinetic parameters in plasma, after administration of venlafaxine extended release (75mg) and desvenlafaxine (100mg) to healthy EM and PM subjects [18].

CYP2D6 status	C_{max} (ng/mL)	T_{max} (h)	AUC (ng*h/mL)	$t_{1/2}$ (h)
VEN administration (75mg)				
VEN pharmacokinetics parameters				
EMs	39.6	6.0	591	10.9
PMs	98.6	7.0	2548	12.7
ODV pharmacokinetics parameters				
EMs	104.3	10.0	3078	13.6
PMs	23.4	16.0	844	14.4
ODV administration (100mg)				
ODV pharmacokinetics parameters				
EMs	190.0	8.0	5630	9.51
PMs	249.6	6.0	5992	11.43

Another factor that should be considered related to the efficacy of VEN in treating MDD, is how the co-administration of other drugs interferes with VEN bioavailability and therapeutic

outcomes. Although lots of pharmacokinetic-based drug interactions have been reported in numerous clinical trials, the consequences of it have not been widely explored. However, not all drug interactions have a negative impact on therapeutic results, and some medication may even be intentionally added as a strategy for therapeutic optimization [11].

Considering the co-administration of CYP2D6 inhibitors, there will be an increase of systemic exposure to VEN and a decrease of ODV serum concentrations. That could lead to some side effects increased, like explained before. However, some authors suggested that this interaction does not present substantial implications on therapeutic results due to the demonstrated VEN and ODV equipotency as antidepressant agents and because the total active moiety (VEN plus ODV) concentrations only suffers minimal changes. So, generally, the co-administration of CYP2D6 inhibitors do not require dose adjustments, but there are some CYP2D6 inhibitory drugs like terbinafine that needs extra care if co-administrated with VEN. In this particular case, it was demonstrated that VEN AUC increased 490%, leading to toxic effects. Some other examples of drug interactions involving VEN are represented on Table 3. The inhibition of VEN minor metabolic pathways mediated by CYP3A4, CYP2C9 and CYP2C19 has not been extensively studied. However, these isoenzymes are responsible for synthetize non-active metabolites, so, a reduction of mediated drug clearance could happen which can precipitate the appearance of toxicity [8,12].

Table 6 - Examples of pharmacokinetic-based interactions involving venlafaxine, with scientific evidence based on pharmacokinetic clinical trials.

Drug(s)	Interaction mechanism	Consequences and clinical effects	References
Bupropion	CYP2D6 inhibition	↑ [VEN] in plasma → Serotonin and norepinephrine related adverse effects (anxiety, blood pressure raise)	[19] [20]
Fluoxetine/Norfluoxetine (SSRI's)	CYP2D6 and CYP2C19 inhibition	Serotoninergic Syndrome	[12]
Cimetidine	CYP inhibition	Unknown	[21]
Diphenhydramine	CYP2D6 inhibition	Unknown	[22]
Imipramine	CYP2D6 inhibition	↑ [VEN]; ↓ [ODV]; ↔ ODV+VEN	[23]
Ketoconazole	CYP2C, CYP3A4, and P-gp inhibition	Unknown	[23] [24]
Propafenone	CYP2D6 and P-gp inhibition	Visual hallucinations and psychomotor agitation	[32]
Quinidine	CYP2D6 inhibition	Cardiovascular toxicity	[38]

Haloperidol, Desipramine, Imipramine, Metoprolol and Risperidone	Possible CYP2D6 inhibition	Once these drugs are metabolized by CYP2D6 too, there will be an ↑of their concentrations and ↑of their side effects	[19] [32] [37] [38] [39]
Indinavir	P-gp induction	Unknown	[11]

A special attention is also required toward VEN co-administration with other CYP2D6 substrates, since VEN has a high bonding capacity to this isoenzyme. If these other substrates have a narrow therapeutic window and do not present other alternative metabolic pathway, extra monitoring and careful in polypharmacy therapy management must be considered [12].

1.1.2 Barriers and other constrains to reach the brain

As already said, another issues that need to be considered are the unique characteristics of cerebral natural barriers BBB and BCF, and the transport proteins/channels associated to them. These are the major hurdles to the entrance of many molecules, preventing some drugs and xenobiotic tissue penetration and also their accumulation in cytotoxic levels [25,26]. This also maintains a constant internal environment that is important for optimal neuronal functioning [5]. However, once VEN has its therapeutic targets at the brain, these limitations need to be crossed, aiming a maximum number of drug molecules to reach their specific receptors which leads to better therapeutic outcomes [5].

P-gp is one of the most important resistance transporters related to CNS-acting drugs once it influences their brain concentrations. It belongs to the multidrug resistant protein (MDR) family and is a product of gene expression MDR1 or ABCB1 [5-27]. It is an ATP-binding cassette transmembrane protein responsible for the active efflux of a broad spectrum of structurally unrelated compounds [25,26]. It is present at high concentrations in the luminal membrane of brain capillaries endothelial cells, more specifically in microvessels present at the interface between blood and CNS. It is also present in the apical surface of the epithelia cells that constitute the choroid plexus. Here, it effluxes compounds into the blood, preventing drugs to gain access to the brain tissue [27]. It has a significant effect in drugs absorption, distribution, metabolism and excretion (ADME), especially when oral bioavailability and CNS distribution are considered. The reason for it is that P-gp is not only present at BBB but also expressed at the BCF, intestine, lungs, heart and pancreas [25,27]. Changes in expression and activity of this transporter can dramatically influence the pharmacokinetic profile of drugs that interact with it, resulting in clinical drug-drug interactions [25], due to the induction or inhibition capacity that some drugs have in P-gp expression and also the bonding strength that have to it. Brain drug levels differences and, consequently, the therapeutic outcomes variations between individuals can be observed also because of P-gp genes polymorphisms. All

these factors are limitations to treat MDD and other psychiatric disorders and, in some cases, it even can contribute to potential toxicity for some drugs [5].

Some studies using P-gp knockout mice have been performed to investigate the *in vivo* role of this transporter related to the administration of VEN [5-25-26]. It was shown that VEN is a P-gp substrate, as well as it major metabolite ODV. The affinity to it does not differ between *O*- and *N-desmethylvenlafaxine* but, in what concerns to S- and R-VEN enantiomers, the brain S/R concentration ratios were significantly different when knockout and wild-type mice were compared. This suggests that the affinity of the two enantiomers of VEN to P-gp is different [5]. Some other important point to consider is the P-gp efflux protein expression induction by VEN in brain and intestinal endothelium cells. A prove of it is the reduction of apical-to-basolateral permeability of a drug efflux probe (rhodamine 123) across *in vitro* models of BBB and intestine (Caco-2 cells) [5,25,26]. This causes a drug efflux increase leading to some drug-drug interactions in humans. The impact of this mechanism when is used ODV is smaller [25]. So, it can be considered that both VEN and ODV are P-gp substrates and, contrary to ODV, VEN is also an inducer of it. That is why oral bioavailability and distribution of VEN and ODV can be modified, as well as their therapeutic outcomes (efficacy *vs* adverse effects). In theory, that is also a reason to have caution when another P-gp substrates are concomitantly used in patients receiving VEN, considering that many of them have narrow therapeutic ranges and that VEN can precipitate changes in rate and extend of their systemic exposure [12].

To sum up, a lot of problems have been associated with VEN oral and intravenous (IV) administration routes, influencing its bioavailability and, ultimately, the therapeutic outcomes in treating MDD. So, the need to found new strategies (e.g. nasal delivery) to overcome this issue and improve VEN concentrations on its brain targets, as well as decrease the adverse effects associated, has become a priority nowadays once VEN is one of the antidepressants with a better therapeutic profile currently prescribed to treat MDD and anxiety disorders.

1.1.3 Intranasal drugs administration

One of the options that has been explored in recent years, not only for VEN but also to other drugs whose targets are especially localized in the brain (and other organs too), is the intra-nasal (IN) route of administration. Drugs administrated to CNS by this via not only circumvent BBB but also avoid hepatic first-pass metabolism, systemic dilution effect, reduces drug delivery to non-target sites, enables administration of lower doses and, in turn, reduces toxicity [28,29]. It is also a good alternative because it is non-invasive, safe, it does not require any therapeutic agent modification, it is practical and convenient to administer, accomplishing faster and higher levels of drug absorption and, in the end, it enhances the

therapeutic compliance for some patients, minimizing the requiring dose, especially for those that do not adhere to therapeutic schedules [6,28,30]. Although this method produces less undesirable consequences while still achieving desirable CNS effects, each drug must be individually examined to evaluate their physical and chemical properties that can affect their presence in nasal mucosa, the sense of smell, specific immunity nasal mechanisms, lymphatic's and deep cervical lymph nodes that can drain some molecules, impairing drug CNS entrance. For example, in contrast with the IN delivery of hydrophilic compounds that typically result in low or no systemic exposure while targeting the brain, it can be difficult to avoid systemic exposure with small lipophilic molecules intranasally delivered [28]. That is because this transport route is mainly dependent of the lipophilicity, molecular weight and degree of ionization of the therapeutic molecules [30].

There are many reasons for the nasal route has been considered one of the most reliable administration alternatives nowadays. They are mainly related with the physiological and anatomic characteristics of nasal cavity. Highly relatively vascularized and permeable mucosa's epithelium, porous endothelial membrane, direct access to this organ, large surface area especially for fast absorption drugs, quick onset of action, lower enzyme levels compared with GIT and liver that avoid the drugs degradation or modification, high total blood flow per volume and direct drug transport to systemic circulation and brain are the main reasons to explore it, especially for drugs that are poorly orally absorbed or have to be given by injection (small molecules and macromolecules) [28,30]. Besides, and especially for small lipophilic molecules, when IN route is compared to the IV route, similar plasmatic drugs profiles can be observed [28,30]. However, nasal drug absorption is not only dependent of these characteristics. Small and uncharged particles easily find their way through mucous, whereas large and charged ones find it more difficult. Structural changes can occur in the mucous layer after contacting with the drug formulation, mostly due to environmental factors modifications in there such as pH and temperature. Potential metabolism by enzymes present in the nasal cavity can also arise, which limits drugs residence time in there and, as consequence, their reaching to target receptors [1,31]. An important point reported was that drug concentration increases in blood and CNS with an increase in their octanol:water partition coefficient, but the permeability and surface area in the formulation deposition site may also affect nasal absorption [10,31]. It also cannot be forgotten the role of mucous membrane cilia clearance that is capable of clearing the administrated drugs. As a consequence, the available time for absorption is limited, contrasting with the huge membrane area provided by numerous microvilli on the ciliated epithelium, which greatly enhance drug absorption when compared to non-ciliated epithelium [10]. Talking about how drug distribution in nasal cavity affects the absorption efficiency, it must be considered that nasal particles deposition is related with airflow resistance provided by nasal mucosa. The particles deposition pattern at respiratory tract is also a function of their size, shape, density and hygroscopicity, as well as the pathological conditions in nasal passages. But, it is mainly

the particle size distribution that determines the site of deposition and affects the subsequent biological responses [28].

Pathways involving nerves that connect nasal passages to the brain and spinal cord are also important. Those that involve the vasculature, cerebrospinal fluid (CSF) and lymphatic system have been tested for some molecules passage from nasal cavity to CNS [28].

Nasal cavity, mainly the neuroepithelium in its upper portion, is the only site in human body where the nervous system is in straight contact with the surrounding environment. Drugs uptake from nasal mucosa into the brain occurs by direct passage from olfactory region to the brain. Once the trigeminal nerve innervates all the respiratory epithelium, being this one of the biggest part of the nasal cavity, drugs can also reach brain targets through this nerve. It can also be systemically transported in which part of the drug is absorbed into systemic circulation by diffusion through blood capillaries of the respiratory mucosa while the other part may enter into the blood circulation via olfactory region, particularly at the level of lamina propria [10]. Subsequently, drugs reach the brain by crossing the BBB and CFS, as it can be seen in Figure 3. The pathway followed by each molecule is mainly dependent of their characteristics already described. However, these routes have not been widely investigated in human beings due to difficulties in quantitative measures of drugs in CNS and brain [28-30].

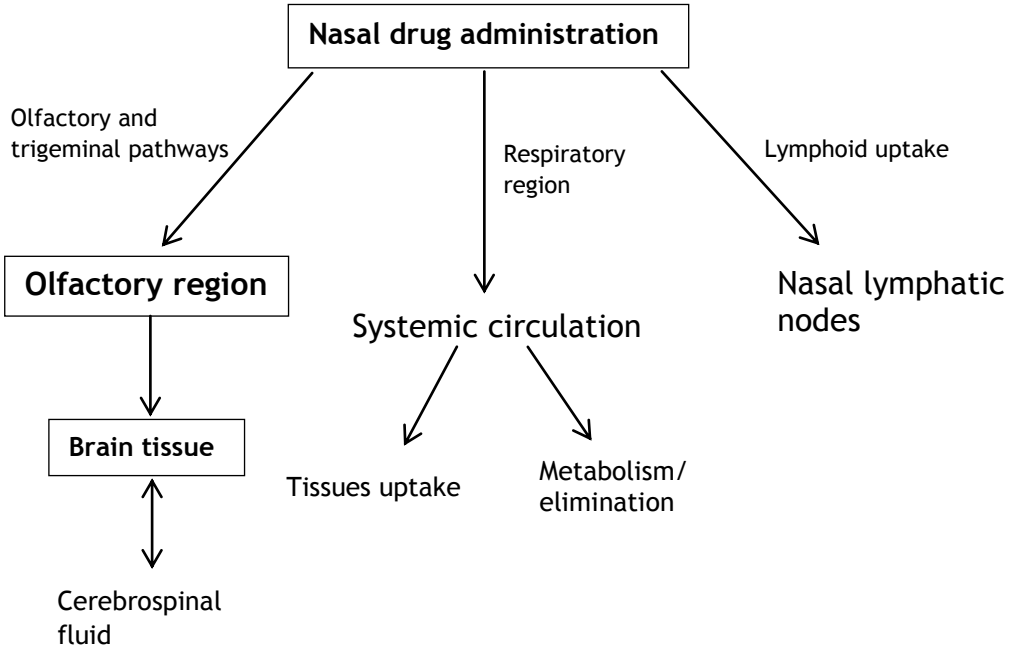

Figure 3 - Routes that a drug can follow after intranasal administration, including the ones that lead to the drug receptor targets and the elimination pathways.

Since it has good permeability, the nasal respiratory route is considered one of the most important sections to systemically deliver drugs, being it a much more attractive alternative to conventional routes. It revealed a higher and rapid drug action onset, especially for those which have poor oral absorption, like proteins and peptides [29,30]. Respiratory mucosa region is the largest part of the nasal cavity and it is where the trigeminal nerve pathway is located, being full of blood capillaries with an abundant bloodstream. So, even though a broad number of drugs can enter into the systemic circulation directly though respiratory region and, subsequently cross BBB to reach CNS, some compounds can gain access to caudal brain via the trigeminal nerve or by lymphatic and perivascular spaces upon lamina propria absorption [10].

Drugs physicochemical properties are factors that must be considered in the evaluation of transport rate and cross capacity from nose-to-brain. From all, three are more relevant for this administration route: the relative molecular weight, lipophilicity and the degree of dissociation. First, most of the small molecular weight (≤400Da) drugs can be freely transported into the brain through nasal mucosa epithelium but, in general, those that have a molecular weight above 1000Da show a poor capability in penetrating the physiological barriers, indicating that the rate of mucosa permeation is highly correlated with molecular size. Second, nasal mucosa is easily penetrated by highly lipophilic drugs with a well-defined linear correlation between the drug's oil-water distribution coefficient and its absorption rate constant. Finally, it is well known that drug concentration in CNS is inversely correlated with its dissociation degree [10].

Still, in what VEN and ODV are concerned and, particularly considering their low oral bioavailability and the brain location of their target receptors, is the olfactory direct route

that mostly matter to investigate in order to overcome all the problems associated with currently used formulations. That is because the olfactory region, next to respiratory region, is the top site from where drugs can be directly delivered into the CNS, in which drug concentrations in olfactory bulbs, after IN delivery, were found to be among the highest in CNS [10,28,30].

Human olfactory mucosa, located in nasal cavity roof, contains olfactory cells (bipolar neurons composed by one pole contacting with the external environment and the other located in lamina propria), entering in olfactory bulbs though the cribriform plate of ethmoid bone. By this way, the IN administrated drugs distribution from the upper part of the nasal cavity is direct to the brain, bypassing the BBB and other barriers [28,30].

Unlike the olfactory nerve, the trigeminal nerve enters the brain through both the pons and the cribriform plate, which allows a drug delivery to both the anterior and posterior brain regions [30].

Generally, substances transportation along the olfactory and trigeminal nerve pathways can happen through both intracellular and extracellular mechanisms. The first one includes the uptake into olfactory sensory neurons within nasal cavity, even by simple diffusion, receptor-mediated endocytosis or adsorptive endocytosis and pinocytosis, being followed by slow axonal transport. These can take several hours/days for the drug to appear in the olfactory bulbs and other brain areas [1,30]. On the other hand, extracellular transport is rapid and likely accounts for much of the quick delivery and onset of action observed with IN CNS therapeutics [30].

To be more specific, drugs that enter in the nose to get into the brain can follow different mechanisms of transport along the olfactory nerve:

- Axonal/intracelular transport: drugs internalization in primary nerves of the olfactory epithelium is done through passive diffusion, receptor-mediated endocytosis or adsorptive endocytosis and pinocytosis, followed by slower axonal transport. This allows the uptake of drug molecules into olfactory receptor neurons. This transportation mechanism takes several hours or days for olfactory bulbs and other brain areas drugs appearance, being the small lipophilic molecules the ones which mainly go through this path to brain [33,34].
- Perineural/extracelular transport: polar molecules rapidly move between nasal epithelium cells tight junctions requiring only several minutes (no more than 30 minutes) for a drug reach the olfactory bulbs and other CNS areas after IN administration. However, due to some structural changes related with depolarization and action potential propagation in nerve's axons, drugs delivery can be blocked or delayed. Besides, the majority of IN administrated drugs rapidly reach brain targets through this pathway, being this one the most promising way for CNS drugs transportation [33,34].

In what trigeminal nerve pathway is concerned, the ophthalmic and maxillary branches of it are equally important for nose to brain drug delivery, once neurons from these branches directly pass through nasal mucosa [1,33,34]. This nerve creates entry points into both caudal and rostral brain areas following IN administration [28]. Interestingly, a small portion of the trigeminal nerve also ends in the olfactory bulbs [1,33]. Since both the olfactory and trigeminal nerves are in contact with the olfactory bulbs, this is what sometimes makes difficult to distinguish what way the intranasally administrated drugs use to reach it and, subsequently, other brain areas. In many cases both could be involved, however more research is needed to prove it [1,33]. Both trigeminal and olfactory delivery pathways are shown next in Figure 4.

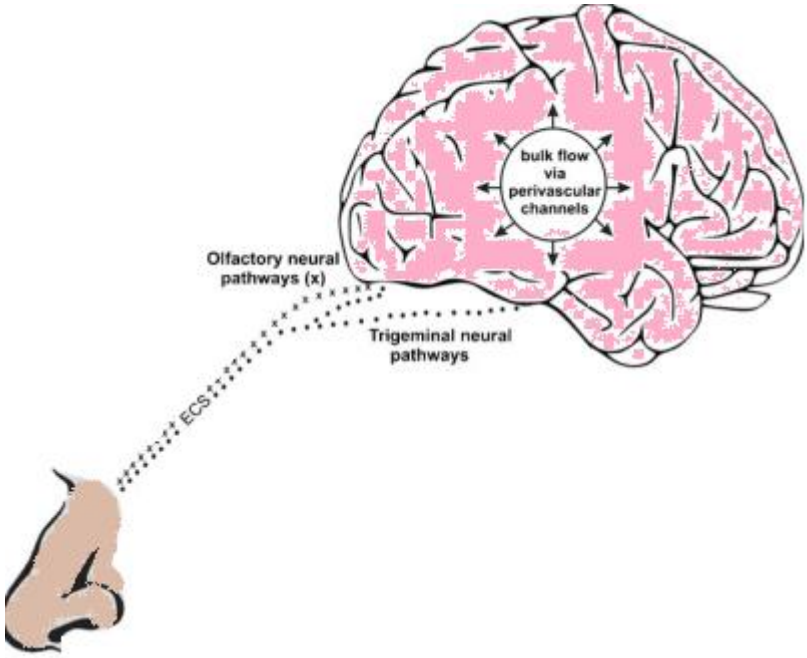

Figure 4 - A scheme illustrating the nose-to-brain mechanism of drug delivery. It can be seen both olfactory and trigeminal nerves routes, in which the first terminates in the olfactory bulbs and, the second one enters at the level of the pons and the cribriform plate, allowing the drug to be distributed to the posterior and also the anterior regions of the brain.

To enhance the IN drug absorption, it is important to adjust drug concentration, dose volume and dosage. Nasal absorption of most drugs increases with concentration increasing, especially for those that penetrate through passive diffusion. Within a certain dosage range, the drug absorption and therapeutic effects would rise with an increased dosage. However, the volume of nasal cavity is limited, being the reason for the nasal administration dose is restricted only to 25-200μL, thus constricting to a certain degree the amount of drug transported from nose to brain [30]. So, due to this restriction, it was needed to adapt the formulation type, in order to promote most efficient drug transportation, even by trying to weak the mucociliary clearance, prolonging drug retention or contact time in the absorption site, or especially by increasing the drug deposition on olfactory mucosa. Additionally, it is important to adjust the pH of nasal formulation to 4.5-6.5, that will result in a better and

efficient drug permeation, as well as local irritation avoidance [31]. All these factors will decide the extension of CNS drug delivery. For example, mucoadhesives are capable of dilating tight junctions and opening paracellular paths, increasing absorption extension. Also the use of penetration enhancers (e.g. surfactants, bile salts,…), enzymatic inhibitors, pro-drugs and novel pharmaceutical dosage forms can increase permeability across nasal mucosa [29-31]. It was already used some kind of IN dosage formulations like drops, powders and aerosols, as well as gelatin, emulsions, ointments, liposomes microspheres, nanoparticles and ion exchange resin preparations. Contrary to conventional formulations (e.g. powders and solutions) that have no mucous membrane adhesion and are easily cleared by the nasal cilia, bio-adhesive polymers, starch microspheres and chitosan preparations have been extensively used to increase the bio-adhesive ability and, consequently, the resident time on the nasal epithelial surface [30]. Micro and nano-dimensional drug delivery vehicles have excellent and broad prospects in the pharmaceutical field. Such carriers systems showed superior outcomes like a greater therapeutic efficacy with a reduced dosing frequency [30].

Now that the most important points about IN administration have been talked about, the main advantages and limitations related to this administration route are summed up in Table 4.

Table 4 - Advantages and limitations of intranasal drugs delivery, when compared with conventional routes of administration (oral and intravenous).

Advantages	Limitations
Drugs direct transport to systemic circulation [10,30,31]	High molecular weight compounds are difficult to deliver for brain [30]
Direct transport to CNS and brain tissues, circumventing the BBB and other barriers to reach the brain receptors [10-28]	Limited formulation volume administration [10,28]
First-pass liver and GIT metabolism bypass, minimizing the administrated dose [10.28]	Mucociliary clearance and ciliary beating reduce drugs resident time [10,28]
Lower enzymatic activity compared with GIT and liver [10,28]	Low permeability of high molecular weight and hydrophilic drugs [10,28,31]
GIT membrane irritation avoidance [10,28]	Nasal enzymatic barriers and presence of mucin hindering the diffusion of drugs [10,28]
Reduced risk of overdose and infection [28]	Lack of studies about IN drugs administration and absorption [10,29]
Patients' compliance increase [10,28]	Minor ratio between olfactory mucosa to all nasal mucosa area [10,33]
Non-invasive administration route [10]	Nasal secretions viscosity, drug solubility and slow diffusion rate through nasal mucosa [10,33,34]
Faster onset of action [10]	Need for special type of formulations to increase bioavailability in brain receptors [30]
P-gp efflux mechanisms decrease [25,26]	Nose kinetic complexity [10]
CNS bioavailability increase, especially for drugs with fairly low brain concentrations [10,33]	
Therapeutic effects enhancement on brain and CNS diseases, with reduction of drugs toxic effects [10,28]	

There are already some nasal drug products for systemic and direct nose-to-brain delivery that are by now on the market or still under development. It must also be noted that an important advance in this investigation field was the synthesis of some nasal drug products for vaccination (e.g.: human influenza vaccine). These are some examples of commercially available IN administration products, being all of them in the form of solution sprays [29]:

- Salmon calcitonin for treating osteoporosis;
- Desmopressin as an anti-diuretic hormone;
- Buserelin for prostate cancer treatment;

- Oxytocin to induce lactation;
- Zolmitriptan and sumatriptan for treating migraine;
- Nicotine for smoking cessation;
- Estradiol as a hormone replacement;
- Oxymetazoline for nasal congestion.

Others are still under development, some only on phase I but others are now on phase II or III of clinical investigation stages. By this time, most of them do not have a specific formulation type. Some examples are [29]:

- Hydromorphone as a solution and morphine, both for pain relief;
- Triptan for migraine treatment;
- Apomorphine to erectile dysfunction;
- Interferon ß for multiple sclerosis treatment;
- Insulin, as a spray solution, to treat type I diabetes and obesity;
- Exenatide for type II diabetes;
- Ketamine, as a solution to acute and chronic cancer pain relief;
- Cyanocobalamin as a nasal spray or a gel, to treat Vitamin B_{12} deficiency;
- Carbamazepine as a microemulsion to treat epilepsy and seizures related to it.

There are also some drugs, still in development phases, whose aim is IN treating of psychiatric disorders. For instance, to treat anxiety disorders and, in a less extend, for amnesia, sedation and seizures, it has been synthesized IN spray solutions of midazolam and lorazepam. Also IN mucoadhesive gel of buspirone has been developed to treat anxiety, alprazolam for panic disorder and triazolam for insomnia treatment. In case of treating psychotic disorders, mostly schizophrenia, acute maniac disorders associated with bipolar disease and other forms of psychosis, it has been investigated a nanoemulsion of risperidone and other for olanzapine, a microemulsion of clozapine and a chitosan/pectin nasal inserts of chlorpromazine [29]. All of these IN formulations have been investigated due to all the advantages provided by IN route, as described above.

In what VEN and ODV are concerned, IN formulations could bring a lot of advantages, leading to an increase in their brain bioavailability. So, once these drugs concentrations in target receptors are higher, serotonin, norepinephrine and, in a lesser extension, dopamine concentrations will increase, mostly in the areas responsible for the development of MDD. In the end, that will increase the success rate in treating MDD and other psychiatric diseases.

Until now, VEN is only commercially available in oral dosage forms which include extended release tablets and capsules, tablets and solutions. If it was developed an IN formulation that could bring great advances in therapeutic MDD outcomes [35]. However, it was already tried a different carrier system to oral administration of VEN. It consisted of polymeric nanoparticles but it has shown some problems like slow onset of action and poor oral bioavailability, even

though the desired outcomes were a quicker onset of action with an enhanced brain uptake [28]. On account of that, there is even one more reason to try some other alternative routes, focusing on IN administration. Of course that the physicochemical properties of both VEN and ODV need to be consider to create the new type of formulation. Some of the most important ones are described below on Table 3.

Table 5 - A summary of the most important physicochemical properties of VEN and ODV to be considered in an intranasal drug formulation type [28,35,55].

Physicochemical properties	VEN	ODV
Molecular weight	277 Da	261 Da
Octanol/water partition coefficient	3.20	2.6
Dissociation constant (pKa)	10.09 for tertiary amine	9.45 for amine moiety and 10,66 for phenol moiety
Water solubility at 25°C	267mg/L	3700mg/L

As it can be seen, both VEN and ODV are tertiary amines with basic characteristics. So, in an environmental condition within a pH range of 5 to 9 (including the blood pH), they are in their cationic form [17-18]. Also, their molecular weight is low and similar, and this property does not greatly influence the passage through biologic barriers. VEN is more lipophilic than ODV, having a greater ability to pass through BBB when comparing to that of ODV. Plus, the water solubility of ODV is much superior to the one of VEN.

Aiming to explore IN administration of VEN and ODV, there must be done suitable pharmacokinetic and pharmacodynamics tests. That will allow understanding if this route increase the efficacy and also if there are differences between cerebral bioavailability after different administration pathways (IV *vs* IN). First of all, it is needed a validated bioanalytical method to quantify these drugs in mouse plasma, brain and liver, however, that does not currently exist. So, in order to perform assays using this administration route for both drugs (VEN and ODV), the development and validation of a reliable quantification technique must be performed. In the majority of published articles that quantify VEN and ODV using high-performance liquid chromatography (HPLC), it is used classic preparation methods like liquid-liquid extraction or solid phase extraction. However, due to some advantages like the number of times that a sorbent can be reused (10-300 times), the apparatus size and its suitability to handle low and higher sample volumes, the low required volumes for conditioning and elution steps, the microextraction by package sorbent (MEPS) it is probably a great approach to analyze VEN and ODV concentrations in plasma, brain and liver after their IN administration [40]. Yet, this method must be validated for these mouse matrices.

The aim of this work was to validate a reliable VEN and ODV quantification method in mouse plasma, brain and liver matrices, since it does not currently exists. This is essential to perform some preliminary VEN and ODV IN administration tests in order to understand if this is a promising route to best treat the referred diseases. If the preliminary studies show good results, hereafter pharmacokinetics, pharmacodynamics and other studies can be performed, always considering the IN administration route its therapeutic advantages.

1.2 Materials and methods

1.2.1 Materials and reagents

Analytical standards of VEN (≥98% lot. 081M4729V) and ODV (≥98% lot. 22M4617V) were provided by Sigma-Aldrich as solid hydrochloride salts. Internal standard (IS) licarbazepine (LIC) was supplied by Tocris Bioscience. Acetonitrile and methanol (HPLC gradient grade) were purchased from Chem-Lab. It was also used triethylamine and orthophosphoric acid, supplied by Fisher Chemical, and sodium di-hydrogen phosphate anhydrous, from AppliChem Panreac. In order to decrease contaminants, ions quantity and interferences in all preparations, it was used ultrapure water (HPLC grade > 18MΩ) obtained through a Millipore Milli-Q water apparatus. The remaining reagents used were Carbopol 974P from Lubrizol, NaCl 0.9%, trichloroacetic acid (TCA) and Pluronic F-127 from Sigma-Aldrich. MEPS 250µL syringe and cartridge containing approximately 4mg of solid-phase silica-C_{18} material (SGE Analytical Science) were also used for samples preparation. To perform IV drugs administration, it was used a 1mL capacity syringe coupled with a metallic needle. For IN administration, it was used a polyurethane tube (24Gx19mm) attached to a microliter syringe (Hamilton Co Reno Nevada).

1.2.2 Stock and intermediate solutions, standards and quality control (QC) samples

All drugs stock solutions were separately prepared in methanol at 1mg/mL. The IS solution was also prepared in methanol but at the concentration of 2mg/mL. From these were individually prepared intermediate VEN and ODV solutions at 100µg/mL and, after it, at 5µg/mL by dilution with methanol. The working IS solution with a concentration of 400µg/mL was prepared through stock solution dilution with water/methanol (75:25, v/v). Appropriate volumes of the intermediate solutions were also mixed and diluted with water/methanol (75:25, v/v) to afford six combined spiking solutions at 0.1, 0.2, 0.5, 1.5, 6 and 10µg/mL for ODV, and 0.05, 0.1, 0.4, 1.5, 6 and 10µg/mL for VEN. After it has been established that the calibration curve for VEN ranged concentrations of 10, 20, 80, 300, 1200 and 2000ng/mL and for ODV ranged 20, 40, 100, 300, 1200 and 2000ng/mL; the combined solutions were used for spiking blank plasma, brain and liver homogenates to prepare the calibration curves solutions.

Note that all working and intermediate solutions were stored at 4ºC in the dark, and the stock solution were stored at -80ºC.

Regarding QC samples, it was established that lower limit of quantification (QC_{LLOQ}) for ODV was 20ng/mL and for VEN was 10ng/mL. According with international validation guidelines (EMA e FDA), the low quality control (QC_1) must be up to 3 times the QC_{LLOQ}, the medium quality control (QC_2) must have a value of 50% the calibration range and the high quality control (QC_3) must have a value of at least 75% the calibration range. So, the values established for QC_1, QC_2 and QC_3 were 60, 1000 and 1800ng/mL for ODV and 30, 1000 and 1800ng/mL for VEN. To prepare the QC samples, four spiking solutions with concentrations of 0.1, 0.3, 5 and 9µg/mL for ODV and 0.05, 1.5, 5 and 9µg/m L for VEN were previously obtained by the same way that those ones used for calibration curves. Then, the QC samples were spiked independently in the three used matrices in order to obtain the required concentrations.

1.2.3 Chromatographic equipment and conditions

The instrumental analysis was carried out through an HPLC system (Shimadzu Corporation, Japan) equipped with a DGU-20A5R automatic degasser, a LC-20AD quaternary solvent pump, a CTO-10AS VP columns oven, a SIL-20ACHT refrigerated automatic injector and a RF-20AXS fluorescence detector. Data acquisition and instrumentation control were achieved by means of Windows 7 LabSolution software.

For separation and detection of ODV, IS and VEN in plasma, brain and liver, it was used the fluorescence detector, once the excitation and emission wavelengths were respectively set at 227 and 300nm. The compounds separation was reached in less than 7 minutes using a mobile phase composed of 10mM phosphate buffer with 0.25% triethylamine (pH 3,3)/acetonitrile (85:15, v/v) isocratically pumped at a flowrate of 1mL/min on a 45ºC thermostated reversed-phase LiChroCART® Purospher Star-C_{18} column (55mm x 4mm; 3µm particle size), purchased from Merck.

1.2.4 Blank matrices

Adult male CD-1 mice aged between 6 and 7 weeks and weighing 30 to 40g were obtained from local certified animal facilities in Faculty of Health Sciences of the University of Beira Interior, Covilhã, Portugal. Mice were housed in a controlled environment with adequate supply of food and water. The environmental conditions establish were 12h of light/dark cycles in a temperature of 20±2ºC and relative humidity of 50±5%.

All the experiments involving animals and their care were conducted in conformity with the international regulations of the European Directive (2010) regarding the protection of laboratory animals used for scientific purposes (2010/63/EU).

Plasma, brain and liver samples used to prepare the validation method solutions were obtained after mice which were sacrificed by cervical dislocation, followed by decapitation. Blood was immediately collected into heparinized tubes while brain and liver tissues were quickly removed, placed in a container with ice and then weighted. To acquire plasma, blood samples were centrifuged during 10min at 4ºC and 4000rpm, and then stored at -20ºC until analysis. Mice brain and liver tissues were homogenized with NaCl 0.9% [41] (4mL per gram of tissue) using an ULTRA-TURRAX® device. After it, tissues homogenates were centrifuged at 17000rpm for 15min at 4ºC, and the resultant supernatants were stored at -20ºC as well.

1.2.5 Sample preparation and MEPS extraction

Each matrix sample (100µL) was individually added with 20µL of IS working solution, 20µL of a water/methanol solution (75:25, v/v), 60µL of ultrapure water and 20µL of 20% TCA aqueous solution in order to precipitate protein content. The mixture was vortex-mixed for 30s and centrifuged at 17000rpm for 3min at 4ºC, being the supernatant transferred to a clean vial after it. The obtained pellet was re-suspended with 50µL of ultrapure water and 2µL of 20% TCA and the mixture was manually mixed and centrifuged using the aforementioned conditions. The resulting supernatant was combined with the previous one and the final volume was subjected to the MEPS procedure.

Before being used for the first time, the MEPS cartridge was activated with 3x200µL of methanol and then conditioned with 2x200µL of ultrapure water. Later, the supernatant volume of the pre-treated sample was subject to three cycles of charge and discharge in the same vial, at a flow rate of approximately 5µL/s. The MEPS cartridge was washed with 200µL of ultrapure water to remove interfering substances and then the analytes were eluted with 200µL of methanol. The methanolic extract was then evaporated until dryness during approximately 8 min at 45ºC under a gentle stream of nitrogen, and after it re-dissolved in 100µL of ultrapure water/acetonitrile (85:15, v/v), vortex-mixed for 30s and injected (20µL) into the chromatographic system. After each extraction and, in order to avoid carry-over effects and to allow conditioning of the sorbent for the next extraction, MEPS sorbent was successively washed with 12x200µL of methanol and 2x200µL of ultrapure water.

1.2.6 Method validation

The present method was validated according to the Food and Drug Administration and European Medicine Agency guidelines [42,43], once some modifications of an already validated method have been done, mostly related with matrices types and experimental procedures. The fundamental parameters herein studied were: linearity, selectivity, precision, accuracy, limits of quantification and recovery.

The method linearity was assessed by constructing calibration curves prepared on three different days for each matrix type, using six calibration standards covering the range of 10-2000ng/mL for VEN and 20-2000ng/mL for ODV. Along with each set of calibration standards,

a blank sample for each matrix (processed without analyte and without IS) and a zero sample (matrix processed only with IS) were also studied. These curves were constructed by plotting VEN/IS and ODV/IS peak areas ratios *versus* the corresponding nominal concentrations in plasma, brain and liver. Data were subjected to a linear regression analysis using $1/x^2$ as weighting factor in the three matrices types and for both VEN and ODV. This was done after it was verified that it existed different variances between the obtained analytical data (heteroscedasticity). This factor was calculated taking into account the plots and the sums of absolute percentage relative error [44].

Selectivity was studied by evaluating the presence of potential chromatographic interferences of matrix components at ODV, IS and VEN retention times. To do that, some blank plasma, brain and liver samples from different mice were analyzed and their chromatograms were then compared with those from spiked matrices samples.

Intra and inter-day precision and accuracy were also evaluated, being the LLOQ defined as the lowest concentration levels that could be quantified with acceptable precision and accuracy. To be valid, the coefficient of variation (CV) values in precision assays must be ≤15%, except for the LLOQ, where it should not exceed 20%. For accuracy, bias values (deviation from nominal concentration) should be within ±15% of the nominal values, except at LLOQ where it should not deviate by more than ±20%. To assess these parameters and evaluate their acceptance, QC_{LLOQ}, QC_1, QC_2 and QC_3 were analyzed in a triplicate way. Inter-day precision and accuracy were evaluated by means of these QC samples analyzed in 3 consecutive days of the assay, whereas the intra-day precision and accuracy data were achieved by analyzing 3 sets of QC samples within a single day.

The absolute recovery of the analytes from plasma, brain and liver samples was investigated at concentration levels of QC_1, QC_2 and QC_3 by testing three replicates of each concentration in each matrix type. The calculations were done by comparing analytes peak areas from extracted QC samples with the corresponding peak area obtained from the direct injection of non-extracted water/acetonitrile (85:15, v/v) solutions at the same nominal concentrations. Similarly, the absolute IS recovery was also determined at the concentrations used in sample analysis.

1.2.7 Preparation of ODV formulation

For IN administration, ODV was previously dissolved in NaCl 0.9%, since it was studied that its solubility in this solvent (between 710 and 725mg/mL) is higher than in water or ethanol. So, ODV was dissolved in NaCl 0.9% at the concentration of 50mg/mL. Then, once the administration dose established was of 0.5mg/kg, 30µL of this saline solution was incorporated in 950µL of the thermoreversible gel and in 20µL of NaCl 0.9%, so that the final concentration was 1.5mg/mL and the total percentage of NaCl 0.9% in the formulation was equivalent to 5%.

Thermoreversible gel for IN administration was prepared based on the cold method described by Scholka (1972) [45] and already used for other investigators that studied IN drug administration [46,47]. To prepare 5mL of this gel, 0.9g of Pluronic F-127, a non-ionic lipophilic surfactant that facilitate solubility of insoluble materials in water [49], must be weighted and then slowly added to 5mL of distilled cold water (5 to 10°C) under constant agitation by means of magnetic stirring. It is important to maintain the water's low temperature by always putting the recipient in ice. The addition of Pluronic F-127 powder must be slow in order to achieve an efficient hydration of each flake. The mixture is then left at 4°C overnight (12-24h) to accomplish a complete polymer dissolution (18% PF-127), having a final clear appearance. Afterwards and according to the technique employed by Badgujar et al. (2010) [48], 0.01g of the mucoadhesive polymer Carbopol 974P (C-974P), which has hydration capacity in order to form colloidal dispersions and a sustained release formulation [49], was weighted and gradually dispersed in the previous PF-127 solution with continuous agitation to promote powder dissolution. A final concentration of 0.2% w/v was reached. Lastly, the solution must be placed in the fridge until a complete dissolution of C-974P is achieved. At this point, a nasal hydrogel formulation composed by 18% PF-127 and 0.2% of C-974P was obtained, exhibiting thermoreversible properties. In fact, PF-127 is a triblock copolymer of poly(ethylene oxide) and poly(propylene oxide) units that is fluid at or below room temperature, but turns to a semisolid gel when the temperature increases as a consequence of the micelle packing disorder-order transition phenomenon [46,47,49]. This thermo-sensible behavior makes the final formulation suitable for gelation into the nasal cavity, providing a sustained residence of the drug at the absorption site.

1.2.8 IN administrations

In the preliminary pharmacokinetic study using an ODV formulation, 5 mice were randomly selected to receive the IN ODV in predetermined and studied time points (30min, 1h, 4h, 12h and 24h).

After animals have been intraperitoneally anesthetized with a pentobarbital solution of 20mg/mL (Ceva Saude Animal), using a volume of 120µL for each 30g of mice weight, ODV was intranasally administrated at the dose of 10µL for each 30g of mice weight (0.5mg/kg). For IN administration, mice were laid down on one side and 10µL of the thermoreversible nasal gel per 30g of mice body weight were instilled using the polyurethane tube attached to the microliter syringe. First, the syringe was filled with an excess of gel, then the tube was attached and, only after it, the formulation volume was adjusted and administrated intranasally. For that, the tube was inserted about 10mm deep into one of the nares, enabling the formulation delivery towards the roof of the nasal cavity. Matrices samples were obtained and conditioned following the same above described procedure that was applied to obtained blank matrices.

1.3 Results and discussion

1.3.1 Optimization of sample preparation and chromatographic conditions

One of the most important steps that must be done in all chromatographic bioanalytical methods is the sample pre-treatment and extraction, in order to separate and isolate the compounds of interest from other matrix endogenous components, like proteins and lipids, that can complicate the analysis and eventually lead to rapid clogging of analytical devices and systems. There must also be considered that some analytes can be bounded with sample proteins, being this another reason to remove them. So, to induce proteins precipitation, prolonging MEPS cartridge life and obtaining cleaner chromatograms in order to allow the quantification of low concentrations of VEN and ODV in samples, it was used 20% trichloroacetic acid before MEPS loading [42]. Also to increase VEN and ODV recovery, after the first centrifugation and supernatant collection, the obtained pellet was manually resuspended using water and this acid, aiming to precipitate the remaining proteins.

Talking about MEPS procedure, it is divided in four steps. First of all, there must be done a sorbent activation and elimination of carryover effect, in this case by using 20x200µL of methanol followed by 2x200µL of ultrapure water. Next, it is done the sample loading step in which the sample is subject to draw-eject cycles. In this case it was studied that, by using 3 draw-eject cycles, it was obtained a good percolation between VEN and ODV and the MEPS sorbent. This is followed by sorbent washing step to selectively remove the interferences from MPES sorbent without significant loss of the compounds of interest. In here, first it was tested 50µL of water [40] however, after trying to do it with 200µL, better absolute recovery results were achieved. Finally, it is done the elution step, in which VEN, ODV and IS are eluted using a polar solvent, in this case, 200µL of methanol. Then, and before the injection into HPLC system, there must be an evaporation step under nitrogen stream (45°C) followed by reconstitution with 100µL of ultrapure water/acetonitrile (85:15 v/v).

According with the VEN and ODV detection method already validated for human plasma [38] and with other published data about VEN and ODV bioanalysis, it was tested some excitation and emission wavelengths included in the fluorescence detection range. In the end, the best defined conditions were 227 and 300nm for excitation and emission wavelengths, respectively [50-51]. Fluorescence detection was always the first choice once both VEN and ODV present fluorescence properties and because these detectors are among the most selective and specific ones used in HPLC analysis. It is also important to refer that fluorescence sensitivity is 10-1000 times higher than that achieved with the UV detectors for strong UV absorbing components, being this another advantage to use fluorescence [52].

With the intent of achieving an appropriate chromatographic separation of the analytes with good peak resolution and within a short running time, adjustments in some analytical conditions were conveniently investigated. Therefore, since VEN and ODV are both polar and

basic compounds, the mobile phase composition and pH were optimized, always considering the reverse phase HPLC characteristics (apolar hydrophobic stationary phase and polar hydrophilic mobile phase). So, the mobile phase selected was a 10mM sodium phosphate buffer/acetonitrile, 85:15 (v/v) at pH 3.3, isocratically pumped at a flow rate of 1mL/min. This pH value allows that higher VEN and ODV quantities are in their ionized hydrophilic form, decreasing compounds elution time and, consequently, whole run time, once there are not so many interactions with the column hydrophobic stationary phase.

1.3.2 Method validation

Chromatographic separation and selectivity

Blank mouse plasma, brain and liver samples were tested and, when compared with chromatograms of samples containing the tested drugs, no interferences were found at the retention times of VEN, ODV and IS, as it can be seen in chromatograms bellow (Figures 5, 6 and 7). Chromatographic separation was reached under the conditions above described. ODV was the first eluted analyte, followed by IS and then by VEN, with retention times of 2, 5.5 and 6.9 minutes, respectively.

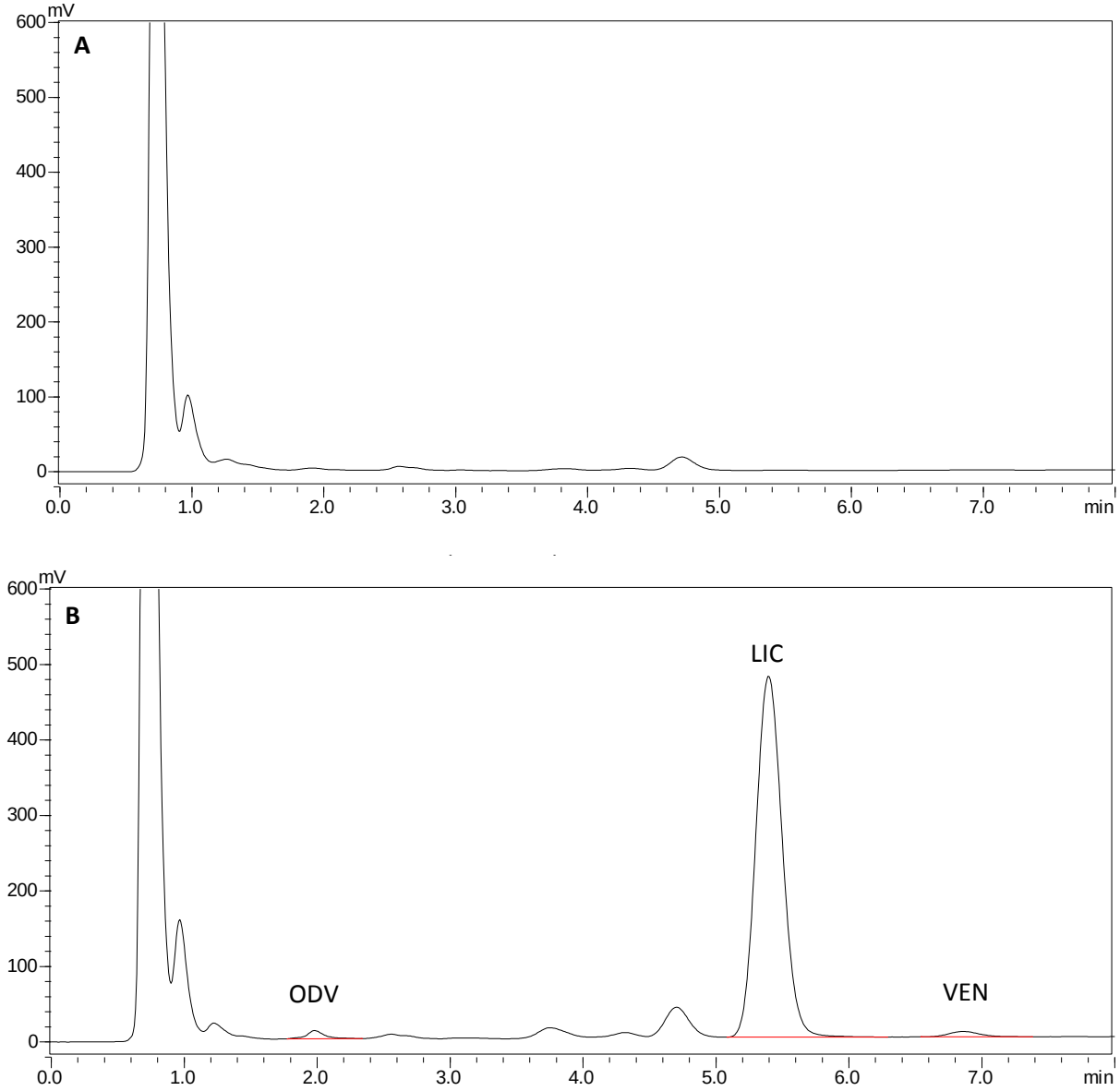

Figure 5 - Typical chromatograms of extracted mouse plasma samples obtained by the MEPS/HPLC method developed: blank plasma (A); plasma spiked with IS licarbazepine (LIC) and the analytes Desvenlafaxine (ODV) and Venlafaxine (VEN) at concentrations of the lower limit of quantification (B).

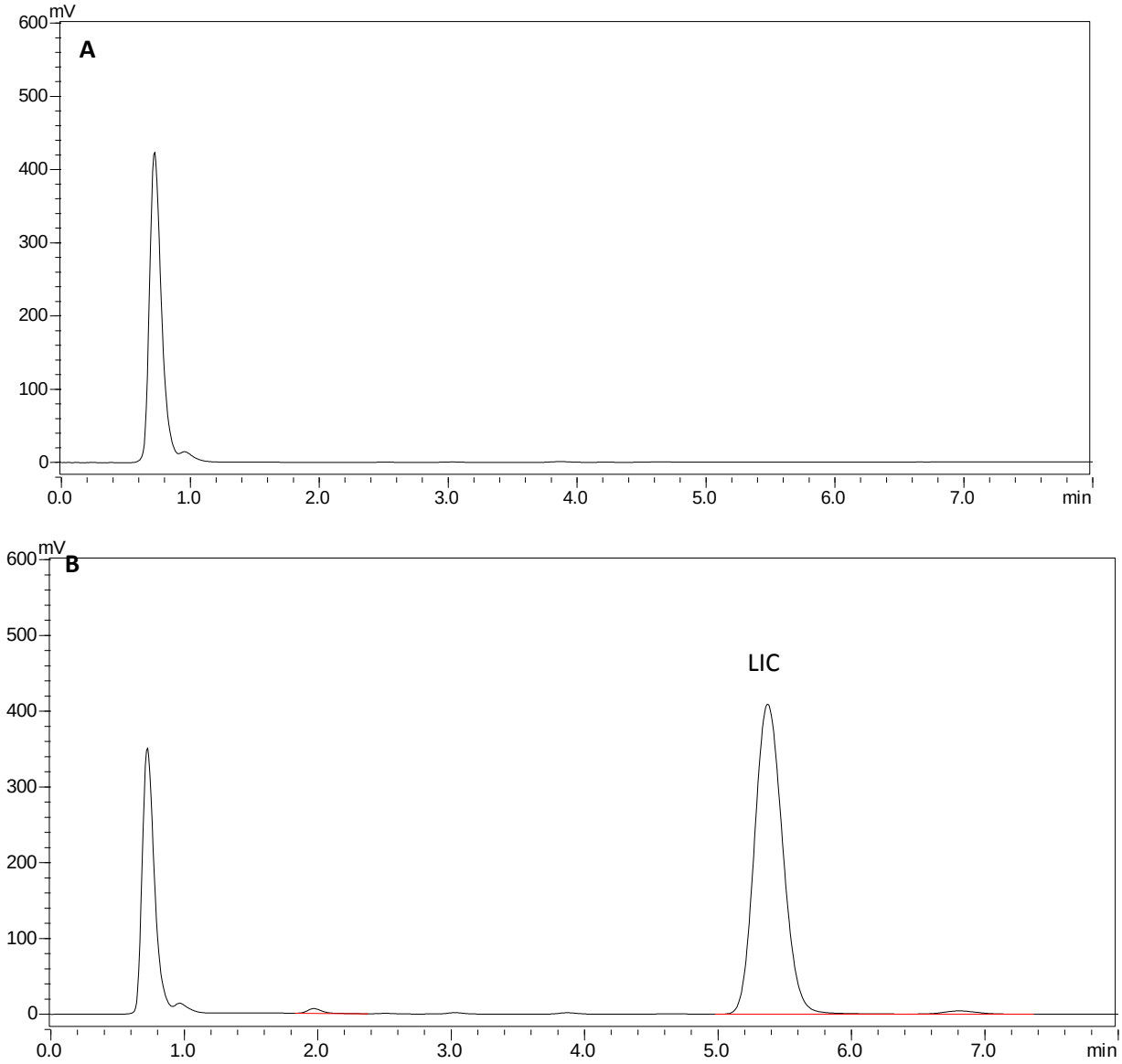

Figure 6 - Typical chromatograms of extracted mouse brain samples obtained by the MEPS/HPLC-FLD method developed: blank brain (A); brain spiked with IS licarbazepine (LIC) and the analytes Desvenlafaxine (ODV) and Venlafaxine (VEN) at concentrations of the lower limit of quantification (B)

ODV VEN

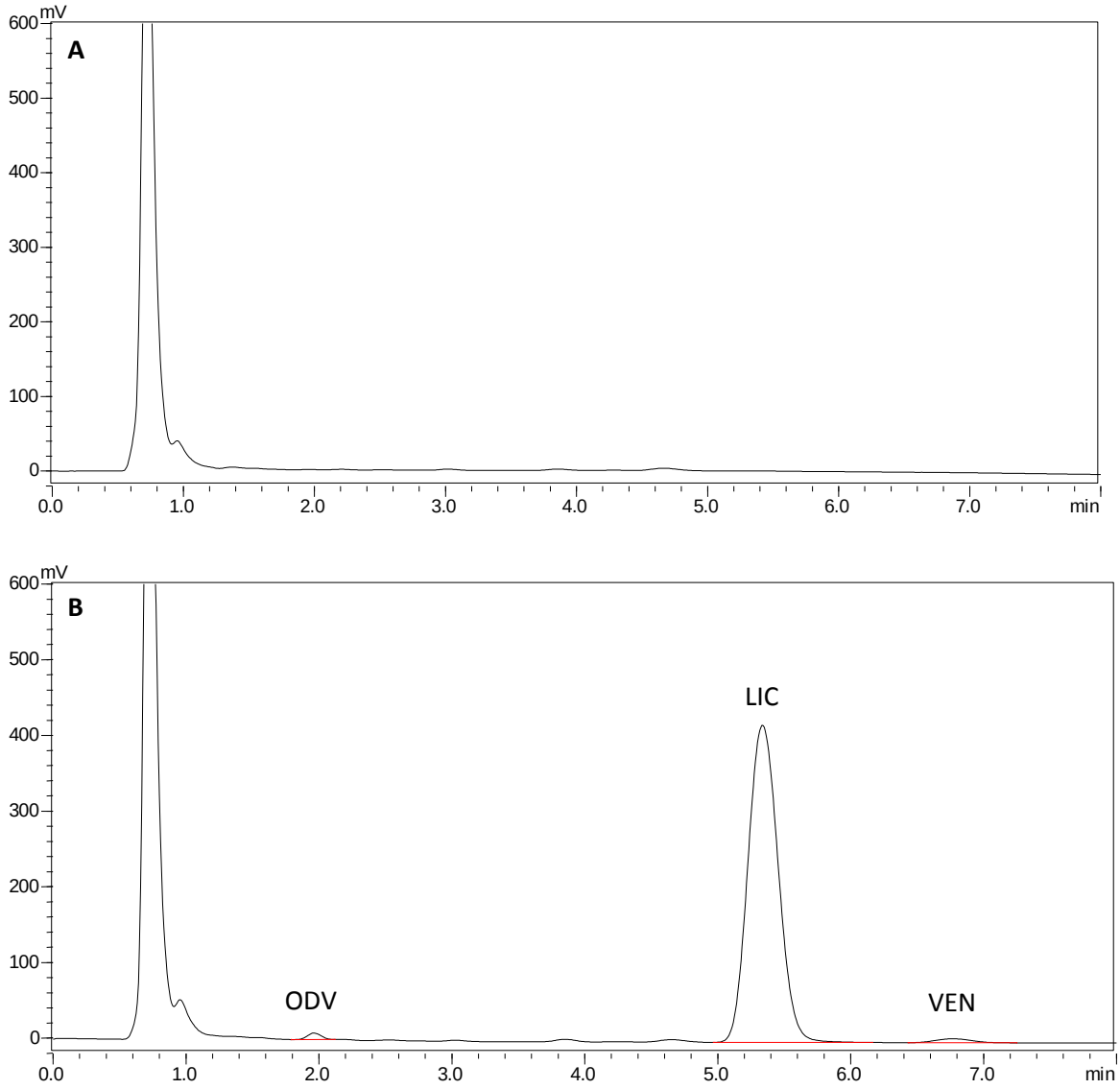

Figure 7 - Typical chromatograms of extracted mouse liver samples obtained by the MEPS/HPLC-FLD method developed: blank liver (A); liver spiked with IS licarbazepine (LIC) and the analytes Desvenlafaxine (ODV) and Venlafaxine (VEN) at concentrations of the lower limit of quantification (B).

Linearity and LLOQ

To be considered a linear method, it is establish that coefficient of determination (r^2) must be ≥ 0.98 [42,43]. So, evaluating the obtained results after mouse plasma, brain and liver samples analysis (Table 5), it can be concluded that the calibrations curves were linear over the concentration range of 10-2000ng/mL for VEN and 20-2000ng/mL for ODV [40,54]. After applying the homoscedasticity test, since it has been established wide calibration ranges for both drugs, it has been concluded that the best-fit weighted factor for VEN and ODV was $1/x^2$. The weighted regression's equations of the calibration curves for the three different matrices studied are also shown in Table 5.

Table 6 - Linearity method demonstration in mouse plasma, brain and liver samples for both Desvenlafaxine (ODV) and Venlafaxine (VEN). Y represents the analyte/IS peak area ratio, x represents analyte concentrations and r2 represents coefficient of determination.

Matrix Type	Analyte	Calibration curve equation (y=mx+b)	r^2
Plasma	VEN	$y=0.00160x+1,54e^{-3}$	0.9980
	ODV	$y=0.00055x+7,84e^{-4}$	0.9978
Brain	VEN	$y=0.00115x+5,83e^{-4}$	0.9975
	ODV	$y=0.00035x+8,35e^{-4}$	0.9981
Liver	VEN	$y=0.00148x+8,57e^{-4}$	0.9968
	ODV	$y=0.00048x+3,42e^{-4}$	0.9974

Considering that the therapeutic window for VEN is 70-300ng/mL and for ODV is 200-500ng/mL [40], it can be quickly concluded that both these values are above the LLOQ established for VEN (10ng/mL) and for ODV (20ng/mL). As it can be seen in Table 2, both values showed good precision and accuracy in the three analysed matrices, once, in all cases, the LLOQ CV values are under 20% and the LLOQ *bias* values are between -20% and 20%. The worse value obtained was for ODV LLOQ accuracy in brain intra-day analysis (*bias*=17.45%), nevertheless it is satisfactory and fulfils the acceptance criteria recommended by the international guidelines. Thus, it can be concluded that this method can be applied in pharmacokinetic and other quantification/analytical studies.

Precision and Accuracy

According with international guidelines, the acceptability criteria for these parameters determination (except for LLOQ values) is a CV<15% for precision and a bias value of ±15% for accuracy. The obtained data for intra- and inter-day precision and accuracy are reported in Table 6. All the matrices samples analysed were in accordance with these criteria. For plasma, the intra- and inter-day CV values did not exceed 7.59% and bias values varied between -0.13 and 13.86%. For brain, and also regarding intra- and inter-day values, the higher CV value obtained was 11.46% and bias values were between -0.65 and 8.15%. Finally, for liver samples, and applying the same determination parameters, CV values did not surpass 10.56% and bias values varied between -5.75 and 3.84%. Therefore, the HPLC method herein developed is reliable, accurate and reproducible over the wide calibration ranges proposed for both VEN and ODV.

Table 7 – Inter- and intra-day precision and accuracy for the determination of Desvenlafaxine (ODV) and Venlafaxine (VEN) in mouse plasma, brain and liver samples (n = 3) at the concentrations of the QCLLOQ, QC1, QC2 and QC3 concentrations of the calibration ranges.

Matrix Type	Analyte	$C_{nominal}$ (ng/mL)	$C_{experimental}$ (mean ± SD, ng/mL)	Precision (% CV)	Accuracy (% *bias*)
	Inter-day				
	VEN	10	10.734 ± 1.211	10.36	7.34
		30	31.185 ± 2.438	7.59	3.95
		1000	998.738 ± 66.698	6.67	-0.13
Plasma		1800	1862.738 ± 41.693	2.24	3.49
	ODV	20	21.187 ± 3.395	12.92	9.08
		60	66.704 ± 6.303	9.25	11.17
		1000	1019,645 ± 84.390	8.26	1.96
		1800	1907.473 ± 50.787	2.66	5.97
	Intra-day				
	VEN	10	10.416 ± 0.181	1.66	4.16
		30	31.775 ± 0.307	4.38	5.92
		1000	1056.397 ± 12.115	0.58	5.64
Plasma		1800	1901.276 ± 25.207	0.63	5.63
	ODV	20	22.474 ± 1.055	4.85	12.37
		60	68.318 ± 0.582	0.86	13.86
		1000	1134.630 ± 12.314	1.09	13.46
		1800	2026.305 ± 52.26	2.58	12.57
	Inter-day				
	VEN	10	10,165 ± 1.462	13.70	1.65
		30	30.318 ± 3.534	11.46	1.06
		1000	1024.929 ± 53.243	5.19	2.49
Brain		1800	1899.33 ± 113.150	5.96	5.52
	ODV	20	21.004 ± 2.108	9.02	5.02
		60	62.520 ± 6.179	9.52	4.20
		1000	1032.751 ± 70.576	6.82	3.28
		1800	1918.28 ± 134.609	7.01	6.57
	Intra-day				
	VEN	10	11.668 ± 0.171	1.56	16.68
		30	32.444 ± 0.359	4.61	8.15
		1000	996.434 ± 28.4	6.27	-0.36
Brain		1800	1788.304 ± 60.111	1.62	-0.65
	ODV	20	23.49 ± 0.174	0.75	17.45
		60	64.823 ± 0.508	0.79	8.04
		1000	1004.089 ± 28.423	2.83	0.41
		1800	1810.861± 5.997	2.87	0.60

	Inter-day				
	VEN	10	10.652 ± 0.071	0.63	6.52
		30	30.549 ± 0.897	2.88	1.83
Liver		1000	986.977 ± 32.394	3.28	-1,30
		1800	1816.833 ± 65.513	3.60	0.94
	ODV	20	21.091 ± 2.669	12.24	5.45
		60	62.304 ± 6.653	10.56	3.84
		1000	981.388 ± 70.826	7.21	-1.86
		1800	1770.55 ± 143.277	8.09	-1.64
	Intra-day				
	VEN	10	10.735 ± 1.040	9.59	7.35
		30	30.969 ± 0.569	2.53	2.32
		1000	962.506 ± 8.489	0.59	-3.75
Liver		1800	1750.772 ± 16.076	2.52	-2.73
	ODV	20	20.272 ± 1.123	4.62	1.36
		60	58.548 ± 1.036	1.66	-2.42
		1000	942.521 ± 13.646	1.44	-5.75
		1800	1718.468 ± 43.984	2.55	-4.53

Absolute Recovery

Both VEN and ODV absolute recovery values for mice plasma, brain and liver samples are shown in Table 7. This data analysis reveals that the mean recovery varies between 64.7 and 53.8% for VEN and between 41.0 and 55.2% for ODV. The correspondent calculated CV values were relatively low. On the other hand, the IS mean recovery in plasma was 55.4%, with a CV of 2.5%, in brain it was obtained a recovery value of 63.5%, with a CV value of 3.22%, and finally in liver the recovery value achieved was of 56.5%, with a CV of 1.94%. These results are not the best when compared with other published validated methods for VEN and ODV detection in human plasma, however, once MEPS is a miniaturized version of the Solid Phase Extraction method and once these matrices are different and more complex when compared with the correspondent human ones, these points could be the justification of the lower extraction efficiencies. Nevertheless, this method is reliable and can be surely applied in studies that use these drugs.

Table 8 - Absolute recovery (%) of Desvenlafaxine (ODV) and Venlafaxine (VEN) from mouse plasma, brain and liver samples at QC1, QC2 and QC3 concentrations of the calibration ranges (n = 3). SD represents Standard Deviation, CV represents coefficient of variation.

Matrix Type	Analyte	$C_{nominal}$ (ng/mL)	Recovery (%)	
			Mean ± SD	CV (%)
Plasma	VEN	30	57.4 ± 3.5	6.07
		1000	64.7 ± 0.4	0.58
		1800	64.1 ± 2.0	3.14
	ODV	60	50.5 ± 0.2	0.43
		1000	55.2 ± 0.7	1.20
		1800	54.6 ± 1.9	3.50
Brain	VEN	30	60.4 ± 1.8	2.91
		1000	55.8 ± 3.9	7.01
		1800	53.8 ± 3.4	6.24
	ODV	60	45.4 ± 1.5	3.32
		1000	42.3 ± 2.8	6.72
		1800	41.0 ± 2.4	5.73
Liver	VEN	30	57.4 ± 1.9	3.29
		1000	55.8 ± 0.5	0.88
		1800	56.3 ± 2.5	4.47
	ODV	60	44.0 ± 1.1	2.52
		1000	41.4 ± 0.4	1.04
		1800	42.0 ± 2.5	6.01

Preliminary IN administration study

Due to some time and available drugs quantity constrains, it was only possible to perform a preliminary ODV IN administration, in order to demonstrate if this administration route has some therapeutic value and, consequently, if all the previous advantages described are fulfil.

There were only tested 5 animals at predetermined time points of 30min, 1, 4 12 and 24 hours. All of them received the IN thermoreversible gel formulation described, in a dose of 0.5mg/kg. After these set times, animals were sacrificed in order to proceed to samples analysis and ODV quantification applying the developed validated method in this work.

Once the administrated concentration was very low and the method ODV LLOQ established was of 20ng/mL, this drug was only quantifiable in plasma samples after 30 minutes and 4 hours of IN administration. The obtained concentrations were of 43.25 and 23.59ng/mL, respectively, not reaching the therapeutic values previously referred (200-500ng/mL [40,53,54]). After 1 and 12 hours, ODV is detected in plasma but it cannot be quantified by using this method. The same happened for brain and liver samples in all studied times (Figures 9 and 10).

Even though, it can be concluded that IN administration route will be promising in future studies using higher concentrations, since with such a low concentration, ODV can be detectable and, in some cases, quantifiable. However, beyond the adjustment of the administrated concentration, some other changes must be done, mostly in what formulation is concerned. For example, some published studies have used propylene glycol to dissolve and further incorporate the studied drugs in thermoreversible nasal gel, obtaining not only good quantification but also good pharmacokinetic results [46]. So, instead of using NaCl 0.9% as it has been done in this work, some other excipients like propylene glycol can be tested. However, the ODV administrated dose can also be increased at least 14 times using a formulation with NaCl 0.9% as the one prepared due to this drug solubility on NaCl 0.9%.

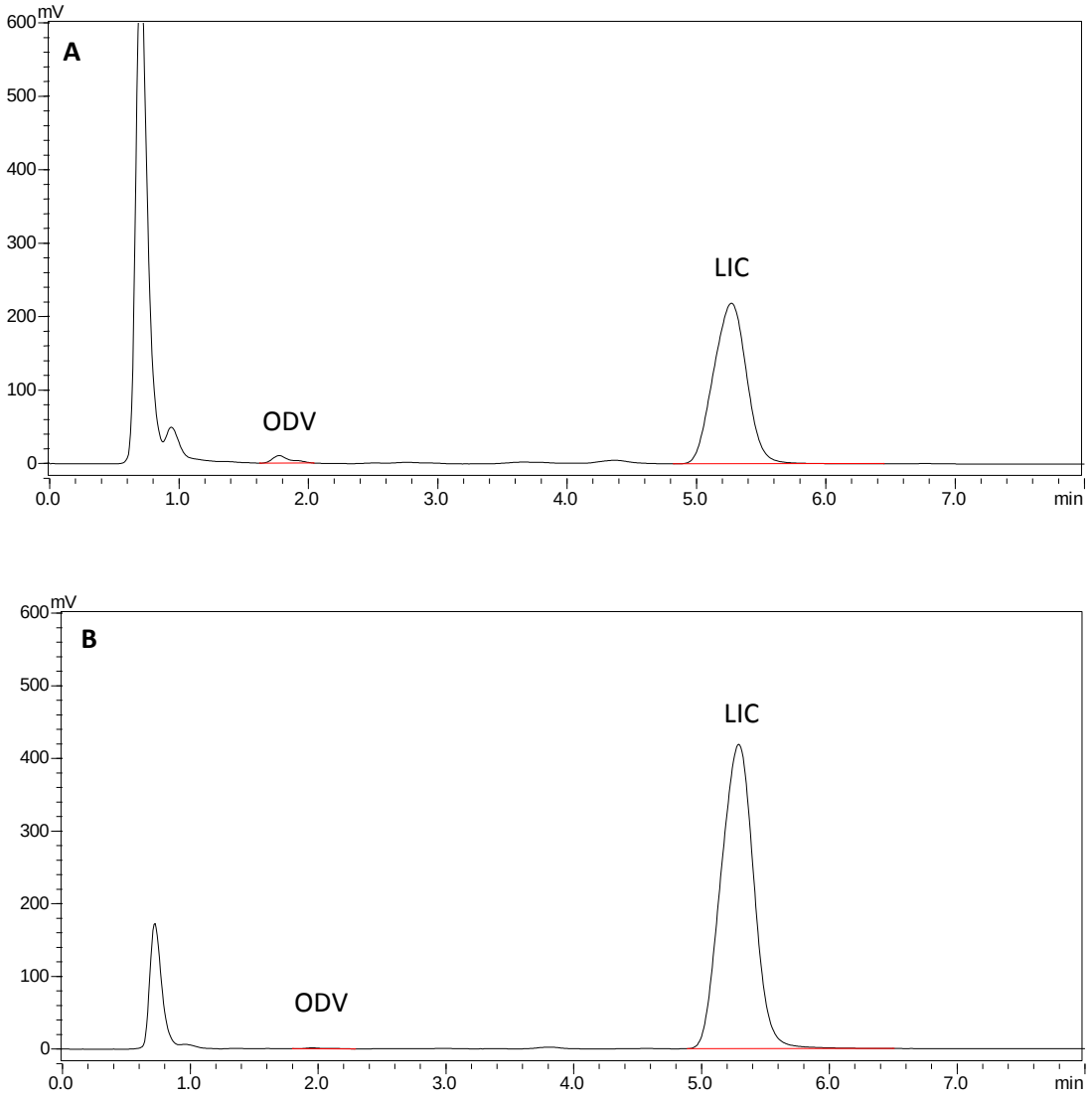

Figure 8 - Typical chromatograms of extracted mouse plasma (A) and brain (B) samples after 30 minutes of the IN formulation administration.

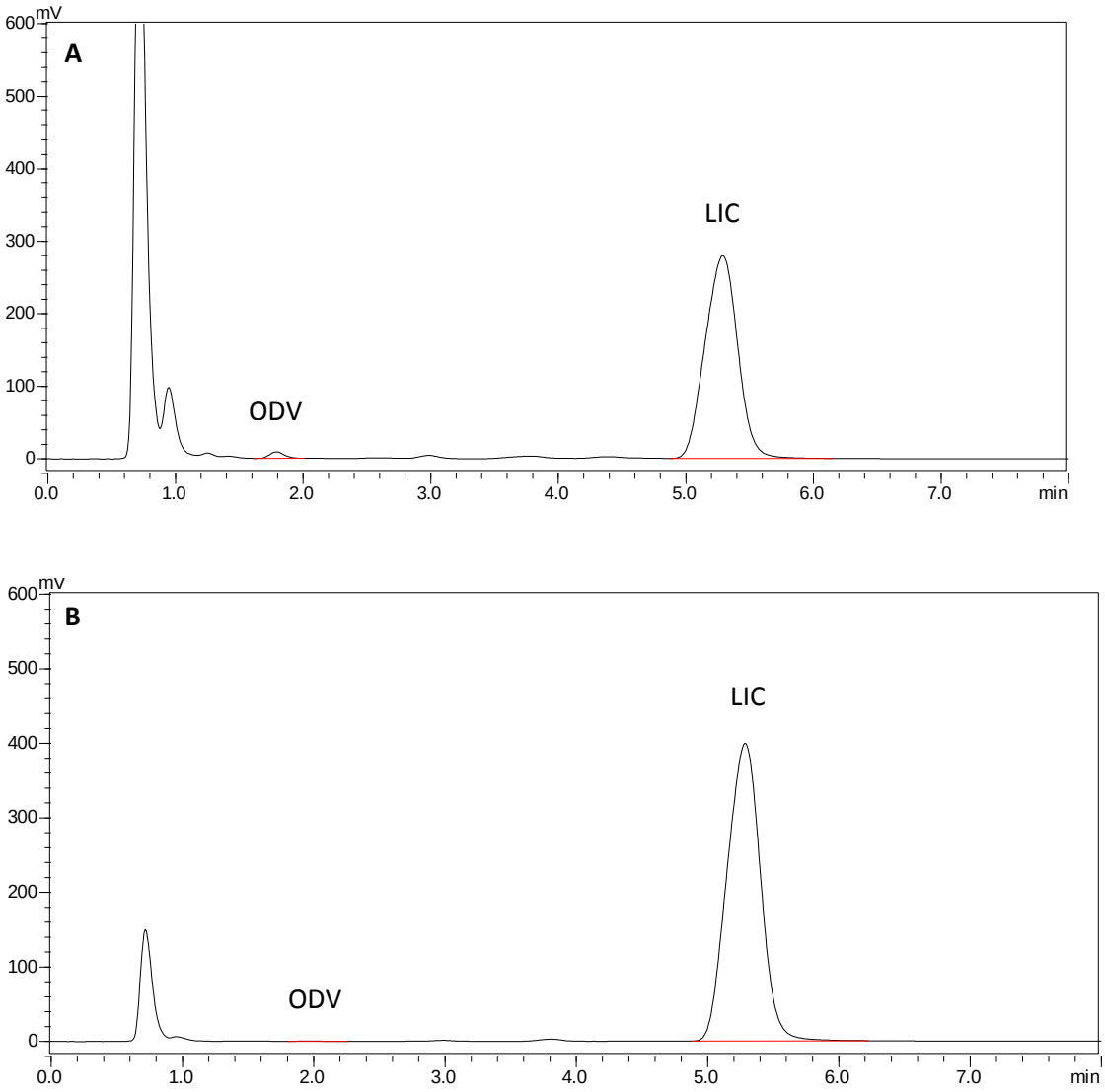

Figure 9 - Typical chromatograms of extracted mouse plasma (A) and brain (B) samples after 4 hours of the IN formulation administration.

1.4 Conclusion

A new and reliable HPLC technique using fluorescence detection and MEPS extraction as an innovative sample preparation method for quantification of VEN and ODV in mouse plasma, brain and liver samples, has been developed and adapted from another already published methods for human plasma. This method presents all the bioanalytical advantages described, being also cost-effective, easy and fast. So, for future pharmacokinetic, pharmacogenomics, toxicological and another kind of studies, and aiming at investigating VEN and ODV IN administration using higher concentrations in mice, this method is not only attractive but also a promising alternative to other common methods that have not all these advantages.

Chapter 2 – Community Pharmacy

2.1 Introduction

My first internship was in the community pharmacy area, more specifically, in Pharmacy Alameda (PA). I have started it in January 26th and I have been there for three months, ending it at April 17th.

2.2 Pharmacy Alameda

2.2.1 Pharmacy structure

Like all pharmacies should be, PA is exteriorly well identified with a green lightened cross and panels showing the pharmacy name. At the entrance, some diverse information like the pharmacy name, its schedule and service days, together with the technical director pharmacist's name and the owner's name is posted.

Talking about the pharmacy interior, PA has only one floor with a huge extended area that covers all the pharmacy's needs. Nowadays, one of the entries is exclusively for the delivery of all the daily orders, being linked with an improvised storage area for stuff like boxes, branches exhibitors, merchandise devolutions to distribution enterprises and product breaks. However, hereafter, it is expected that this space will be restructured, aiming to create some service cabinets and rooms for diverse services and other proposes. But, until then, it can be considered an inactive space connected with the other pharmacy areas by two corridors, one of which directly to the pharmacy commercial area and the other to the backstage areas that are not public accessible. In these last ones, there are located some crucial spaces like the meeting room, containing a small library; staff bathrooms and locker rooms; a private office; a laboratory; a storage sector for surplus like oral, hair and intimate hygiene products, dermocosmetic products and some Over-the-counter (OTCs) drugs that are replaced when it is needed; the fridge, vital to store insulins, some eye drops, some veterinary drugs, vaginal rings, vaccines, injectable drugs, enzymes and other refrigerated products; the filing cabinet and, finally, the orders reception area, directly linked with the robot medication supplier entry. The service area, where the patients are attended, is specially designed for them to feel in a space truly created and dedicated specially for them and their needs. Each sector is well organized, allowing patients to explore and concentrate in their areas of interest. For that, all the colors, products diversity and the way that are exposed, along with the service quality and the staff sympathy, is hardly thought, willing to provide the best satisfaction experience. Now, to be more specific, the patient attending can be done in one of the five service counters, being one of them specially elaborated for sitting attending. This one is aimed for the elderly, patients with mobility problems or for some cases that require more

time and privacy in its approach. In the public area there are also shelving with dermocosmetic and hair products, OTCs, placed behind the service counters in both shelving and lockers and not directly accessible by patients, orthopedic products, medical devices, specially designated for aerosol and drugs inhalation, and some childcare, podiatry and geriatric products. Also for public provision, it is available an equipment with functions for weight, high, body mass index (BMI), blood pressure (BP) and heart rate measure, two private rooms, one for glycemic, triglycerides, cholesterol and other biological parameters determinations, for vaccine administrations and also intended for pharmaceutical counseling. The other one is designated for nutrition, dermocosmetic, audition, podiatry and diabetic's feet appointments. Some of these services need previous schedule by the patients. Finally, it is also available a bathroom that patients can use.

It is important to tell that the majority of drugs are placed inside the robot. However, due to the dimensions of some products, the OTCs seasonality/turnover or even some peculiar product characteristics (e.g. disinfectant and cleaning material), they are placed in drawers behind the service counters. For all cases, the placement identification is registered in each product file integrated in Sifarma 2000 software.

2.2.2 Human resources

Due to the recent PA administration reformulation, there are not yet defined tasks for each pharmacist, however, as it is legally required, there is a pharmacist technical director (TD). It also has three other pharmacists that are the new owners of PA too, one of them being specialized in the dermocosmetic area. They are also the main responsible for the pharmacy management, organization and product buying. To complete the team, there are three pharmacy technicians and a cleaning lady, responsible of daily keeping the pharmacy hygiene required parameters. Besides this team that is present every day in the pharmacy, there are also other professionals that are responsible for specific services in the pharmacy like a nutritionist, a podiatrist and a nurse, although they are only present in scheduled days. In the future, each staff member will have specific responsibilities in specific pharmacy areas or services and, with it, will be also responsible for spreading all guides and information related with his area of expertise to the other staff members. In the end, it will allow them to work as a cohesive team. According to the Portuguese Legislation (PL) [1] the TD duties are:

- Have the responsibility for the pharmaceutical acts done in the pharmacy;
- Patients elucidation and a rational drug use promotion;
- Ensure that prescription-only medicinal products are rendered to patients without prescription only in cases of extremely need, if well justified;
- Ensure that drugs and medicinal products are maintained in a well preservation state;
- Guaranty the hygiene parameters of the facilities as well as a good security in there;
- Ensure that the pharmacy has a sufficient drug provision;
- Continually certify the good tidiness and hygiene of all the pharmacy staff;

- Check that the Ethics Code is respected by all involved in the pharmaceutical activity;
- Assure that all the principles and duties present in the PL are respected.

Analyzing this duties list, it can be concluded that a good TD must be a good team leader, self-preserving not only the pharmacy interests but also and mainly the patients. In the PA case, it can be said that some of these points are not fully delegated by TD but also by both the pharmacy owners that are also pharmacists and the leaders of all the staff. Beside the current pharmaceutical duties, they also have all the commercial and financial responsibilities as well as sufficient product provision, stock and prices management, and staff continual education, formation and motivation.

In order to follow Pharmaceutical Deontological Code [2] and to ensure an updated scientific knowledge of the staff, the owners are currently implementing training courses and distributing flyers about various new themes and products present in the pharmacy, in order to ensure the best pharmaceutical counseling and good therapeutic results for all patients. During my internship, I had the opportunity to assist to some of those training courses about "Caudalie", "Lierac" and "Phyto" dermocosmetic products, "Aboca Laboratories" phytotherapeutic products, and "Philips Avent and Respironics", the brand which supplies baby medical devices and respiratory therapy devices like nebulizers, spacers and holding chambers to the pharmacy. However, in my opinion, the training course that most beneficiated me was the one entitled "Holon Attending", that took place in "Hotel D. Maria", with 2 days duration. In there, I have learned some psychological approaches and strategies that, during my internship and in the future, will help me to understand what are the patients' health problems and how to solve them, always with sympathy, empathy, professionalism and by showing confidence in what I am saying to patients and, at the same time, comprehend their social, health and financial state and affordability. Also, almost at the end of my internship, I had a special training course related with the new electronic prescription system that is being implemented in all Portuguese pharmacies throughout this year. In the future, when the experimental time passes and this prescription type has been totally established through each patient citizen card use, this will allow paper prescription extinction and a total process simplification with mistakes minimization. However, for now, since the electronic readers had been installed by a Glintt technician in the same day of the training course, the staff is only trying this kind of prescription by reading the two fully designed codes contained in all prescriptions that are fully designed for the electronic readers, in order to get familiarized with this system.

I also had the opportunity of doing some research works. One was about drug induced photossensibility (Annex I), aiming to be a currently alerting tool about some ordinary drugs that can cause this adverse reaction, as well as trying to be instructing about how to counsel patients to prevent and treat it. The other one was about inhalation devices (Annex II) and their respective inhalation techniques. I also did a data collection about what kind of inhalation devices were available in the pharmacy, aiming to everyone being informed about

it before the Chronic Obstructive Pulmonary Disease (COPD) and asthma pharmaceutical consultation establishment.

2.2.3 Drugs and health products

First of all, we need to have in mind all the definitions about this theme. Again, according to PL [3] some of these important definitions are:

- Active pharmaceutical ingredient (API): any substance or substance mixture designated to be used in drugs manufacturing, being the one that will have a pharmaceutical, immunologic or metabolic action aiming to restore, correct or modify physiologic functions or establishing a medical diagnose;
- Drug: every substance or substance association with healing or preventive properties for symptoms or diseases in human beings. It can also be administrated with the aim of establishing a medical diagnose or to restore, correct or modify physiologic functions through a pharmacologic, immunologic or metabolic action;
- Reference drug: drug that was authorized based in complete documentation including pharmaceutical, clinical and pre-clinical trial results and information;
- Generic drug: drug with the same quantitative and qualitative APIs composition, the same dosage form and with equal bioequivalence, demonstrated by appropriate bioavailability studies, when compared with the reference drug;
- Officinal formula: any medicinal product that is prepared in a pharmacy according with a pharmacopoeia prescription. It is intended to be supplied directly to the patients served by the pharmacy in question;
- Magistral formula: any medicinal product prepared in a pharmacy according with a medical prescription to an individual patient;

Of great importance too there are the definitions of both psychotropic and narcotic drugs. These ones are defined as any natural or synthetic substance or any natural product included on Tables I, II, III or IV, in case of psychotropic, or on Tables I and II in case of narcotic drugs, according to "*Decreto-Lei n.º 15/93, de 22 de Janeiro*" [4]. For these specific drug groups, it is mandatory that all pharmacies send the log and output registrations of both psychotropic and narcotic drugs to INFARMED (Autoridade Nacional do Medicamento e Produtos de Saúde, I.P). Manly for drugs included in tables I and II, those need to be prescribed alone in a prescription identified as RE (Receita especial) [5]. In the prescription dispense, it is mandatory to make a registration of both patient and acquiring person's data (name, valid identification document number, postal address, phone number, birthday and dispense time) in Sifarma and, in the sale's ending, a ticket which contains a specific code and the designation of "Documento de Psicotrópicos" or "Documento de Estupefacientes" is emitted. Then, together with the prescription copy, they are both filed together in the same file which contains all the other previously referred documents relative to these drug groups. However there are some exceptions, mostly in what benzodiazepines are concerned. It's also important that these

documents be filed in the pharmacy for at least 3 years. To better understand these requirements, all the detailed information is shown in the following Table 9 [6]:

Table 9 - Psychotropic and narcotic documentation and procedures.

Psychotropic and narcotic drugs	Log registration sending	Output registration sending	Balance sheet sending	Prescriptions copy sending
Tables I, II-B, II-C	Every three months	Every months	Annually	Every months but only for manual prescriptions
Tables III and IV, including benzodiazepines	Annually	Not applicable	Annually	Not applicable

Aiming to recognize some drugs of each table, I did a data collection about which of them was in the pharmacy stock. To each table, some examples are:

- Table I-A (narcotic drugs with special prescription): Durogesic (fentanyl transdermal system); MST 1, 3, 6 or 10mg (morphine retard tablets); Oramorph (morphine oral solution); Sevredol (morphine coated tablets);
- Table II-B and II-C (psychotropic drugs with special prescription): Buprex (buprenorphine injectable solution or sub-lingual tablets); Ritalina LA (methylphenidate retard tablets); Transtec (buprenorphine transdermal system);
- Table III (narcotic drugs with normal prescription): Codipront (codeine tablets or syrup); Dafalgan Codeína (codeine coated tablets); Euphon (stirrup containing codeine); Morfex (flurazepam capsules); Olcadil (cloxazolam tablets); Stilnox (zolpidem coated tablets); Valium 5 or 10mg (diazepam tablets); Xanax (alprazolam tablets); etc.
- Table IV (psychotropic drugs with normal prescription): Ansilor (lorazepam tablets); Bialminal and Bialminal Forte (phenobarbital tablets); Bromalex (bromazepam tablets); Cymerion (zolpidem coated tablets); etc.

Since the first day of my internship, I had the opportunity to connect myself with all the drugs and other health products present in PA. The aim was to get familiar and always associate an API, the way that generic drugs are identified, with the brand/reference listed drug product. I was always stimulated to make a correlation between the medical drug prescriptions and a potential disease or diseases that a patient, who presented himself in the pharmacy, could suffer. For that, I was able to consult various scientific information sources like “Prontuário Terapêutico Português 2013”, Sifarma 2000 data base, Infomed and others. All the research was also done so, at the end, I could be capable of distinguish prescription-only medicinal products from OTCs, identify generics, veterinary products, psychotropic and

narcotic drugs, officinal and magistral formulas, phytotherapeutic and homeopathic drugs, dermocosmetic products, special food supplements and medical devices.

I have also realized that a great amount of the medical prescriptions that entered the pharmacy contained psychotropic or narcotic drugs, especially benzodiazepines. On the other hand, nowadays, it rarely appears a medical prescription of even an officinal or a magistral formula, contrary of what happened a few years ago. Despite this, I had the opportunity to prepare a 70% saturated alcoholic solution of boric acid prescribed by a doctor to a child patient with an external otitis, aiming to dissolve accumulated earwax caused by this ear infection. In this case, we prepared it by following the protocoled steps described specifically for this medication in "Formulário Galénico Português" (Annex III), being it the reason to be considered an officinal formula. We have done it in the pharmacy lab, always following all the security requirements, steps preparation and filling the registration sheet requests. In the end, we've packed the solution in a 30mL amber-glass dropper bottle accompanied with its respective label, also performed by us in Sifarma 2000. The label must contain the following information: patient name, drug formulation name ("Solução Alcoólica de Ácido Bórico à Saturação"), drug batch number, expiring date, storage conditions, special instructions for this formulation like "external use" and others, administration route (to this case was topical), dosage, pharmacy identification and TD identification [7]. Finally, the price was calculated accordingly with the legislated parameters and mathematic formulas.

2.2.4 Provision and storage

In what drug cycle is concerned and linking it with all the steps that drugs suffer in a community pharmacy, it is essential for all workers and trainees to know how they come to the pharmacy. For that, understanding which products and suppliers to choose and the criteria to do so is crucial, even more in the actual economic and financial Portugal status and how it affects a pharmacy dynamic. So, these parameters were the first ones that I had contact with. Since the first day of my internship I had started to admit orders. The first thing that I have learned was how to approve an order in Sifarma 2000. The process is quite simple, however, one must pay attention to some details like if the ordered product quantity corresponds with the quantity received, confirm and correct the invoiced and consumer prices, set the trade margins and update all the expiration dates. In this step, it must also be ensured that the products integrity and good appearance are guaranteed during the distribution.

2.2.4.1 Suppliers criteria selection

In PA, the supplier's selection is of the pharmacy owners' responsibility. The chosen ones until now are Alliance Healthcare and Udifar. This was because, after some studies, they have realized that these two were the most reliable due to the number of diary delivers (three and two times a day, respectively), the availability and good conditions of the products required

and received, ensuring the best pharmacy function, but also the buying and negotiation conditions, like bonuses, discounts and portfolio products, and an easy payment. Generally, these orders are performed via Sifarma 2000, however, when it is needed, it can be done by phone or internet. In what OTCs, hygiene, dermocosmetic, pediatric and geriatric products are concerned, the suppliers are usually different and, most of the times, before the order is performed, the owners first meet with a brand delegate to know the commercialized products and to negotiate the best buying conditions. Most of the times, these products are ordered in huge quantities because it allows the pharmacy to benefit of some free products or discounts. I have had the opportunity to be present when some reunions with Pierre Fabre, Philips AVENT and Mustela representatives happened.

Due to the actual Portugal economic situation, there are some drugs, now called "prorated drugs", that are almost sold-out in the pharmaceutical industry warehouses which lead to some stocks ruptures in pharmacies. The pharmaceutical products distributors have a defined monthly stock of these products to supply to each pharmacy. However, if the pharmacies require higher quantities of these products, they can be directly ordered to the respective producing laboratories by an SOS channel. In PA case, that never happened since the required quantity can always be supplied by distributors.

2.2.4.2 The order

Sifarma 2000 has an individual file for each product, commercialized or not, in the pharmacy. In there, it can be found scientific information (e.g. pharmacologic category, formulation type, therapeutic indications, adverse effects, excipients, and drug interactions), brand holder, buying and selling balance, usual supplier and other information. It also contains important data for pharmacy management like pending orders, if the suppliers are exclusive, an actual, minimum and maximum products stock and the location where they are placed in the pharmacy. Relative to products prices, each file also contain information about public selling price, billing prices, margins (%), reference price and a price labels printing option. It's important to refer that the majority of OTC margins are marked by the pharmacy owners. They also manage the minimum and maximum product stocks based in some public consume studied parameters. Some examples are the patients consuming profiles, the most frequent prescriptions and the diseases that they are designated to, the suppliers' bonus, the different year seasons and the products advertisement that are public assessed. Another important tool that Sifarma allows is to know the buying/selling history, mainly through charts. This section also let us know the main supplier, the delivery hours and dates together with the correspondent bill number, the quantity received in those schedules, if the product is sold out, the actual stock, all the previous reference prices, who did the order and its number and kind (manual, diary or instant).

As I have already said, all the orders are done through Sifarma 2000, however, and especially if the network is down or due to an urgent product requirement, they can be done by phone

or fax. In some extreme cases, when a particular product is required by a patient but none of the warehouses have it available, the pharmaceutical or pharmacy technician that is attending a patient requiring it can contact a near pharmacy to ask about their product availability.

Sifarma allows professionals to make two different kinds of orders: the manual and the diary ones. When a product reaches a quantity below its minimum stock established, a diary order is generated automatically to its supplier in a number that allows the reaching of the maximum stock quantity. In case that some product is missing in the diary order, it can be made a manual one through Sifarma, in which it needs to include the product code (that instantaneously converts itself in the name of the product), the quantity required and the supplier's name. Note that the pre-establish supplier marked in the product file can be changed if needed. After a final analysis, the products list is approved and sent to the supplier.

2.2.4.3 Orders' reception and check

Like it was said, a specific number of orders are received every day after they have been approved and sent by staff members. The products are delivered by the distributors in plastic containers predestined for medical products transportation. In those, apart from the order, they also contain a double bill form with the following information: pharmacy and suppliers data, bill number and date, products codes and names, ordered and received quantities, discounts or bonuses, buying and public selling prices, tax percentage (IVA) and the total price. The exception is to non-prescribed products in which their prices are decided by the pharmacy's owners. For the prescribed drugs, the prices are pre-established by INFARMED according with specific legislated mathematical equations based in the drugs production or importing prices in the reference countries (PVA), plus the commercializing (suppliers and pharmacy) maximum margins, the commercializing taxes and IVA (Annex IV). Once that legally exist six different products prices levels [8] – the first one is for products until 5 euros; the second one for products between 5.01 and 7 euros; the third covering prices between 7.01 and 10 euros; the fourth between 10.01 and 20 euros; the fifth for products between 20.01 and 50 euros and finally the sixth for products above 50 euros – there are different corresponding numeric parameters to include in the equation according to each product price level.

In the pharmacy, at the same time that ordered products are introduced in the system through reading their bar codes, it needs to be performed a checking of the received products quantity and analyze if it corresponds to the ordered quantity. Also the billing prices, public selling prices (PVP) and expiring dates need to be checked and corrected. Note that the expiring date that is introduced in Sifarma 2000 is always the lower one for products with the same code. In order to preserve some specific products stability, mainly the refrigerated ones, those are the first to be introduced and then are readily stored. In the end of this

process, if there are some missing products that were ordered, it needs to be performed an informatics communication to INFARMED. Before the products storage, the non-marked products that are publicly exposed are identified with an impressed label containing the correspondent bar code and price, previously calculated based on the pharmacy billing price, the selling margin and the product type and turnover.

It is possible to make devolutions to the suppliers because of the following reasons: market products recalling by INFARMED or pharmaceutic products holders (AIM), products or devices damaged during its transportation, products whose expiring dates are near of ending, products that were not or were wrongly ordered and in cases in which prices were altered or remarked. For that, a devolution note is issued in a triplicate way (two of them go to the supplier and the other remains in the pharmacy), stamped and then signed. All devolutions are electronically communicated to Portuguese financial authority. Then, if the supplier accepts it, it can be regularized by products delivery and/or credit notes. I had the opportunity of making some of these notes for various reasons. One was because, while they were transported, some badly packed alcohol bottles were opened damaging all drug boxes that were in the same container. Other examples were a Vibrocil nasal spray device that did not worked, six received Thyrax pill boxes which expiring dates will end in a month and a thermometer that didn't work at all. Note that in all the devolutions notes, besides the product names, they need to contain the devolution motive and the supplier name.

2.2.5 Storage

In my first internship week, all steps of products storage were explained to me and then I became capable of doing it by myself after some days. The aim was to get familiarized with the robot functioning and to know the place where each product was stored and, sometimes, the reason for it. First, all the refrigerated products need to be put as soon as possible in the fridge, even before we proceed to the orders reception and check. The other ones, after all the steps of the orders reception, have to be stored in a specific place, being most of them introduced inside the robot, particularly all drugs that need a prescription and some OTCs which don't have to be exposed to the public and have a big rotation due to patients knowledge and great use. For that, first we need to introduce the expiring date of all the products individually and then pass their bar codes in the robot code reader. Then, we put them, in a specific position, in the robot's mat to be stored inside it. Note that, due to the form or dimensions of some products or even for their package plastic coating, it is not possible to introduce them inside the robot. Related with expiring dates, it is applied the "First-In-First-Out" policy, in which the products that have a shorted expiring date must be firstly sold. Those need to be stored outside in the drawers linear or exposed in shelving ahead or behind the service counters, according with the product type. It is important to place a particular kind of product in its particular section to maintain all the pharmacy

organization. For example, condoms are placed in the sexuality section; baby dippers in children care section; tooth brushes in the oral hygiene section and so on.

2.2.6 Pharmacy Services

Since PA is one of the Holon Group pharmacies, all the operation parameters of it are identical to the other pharmacies that belong to it. Besides, this group has also a lot of services to offer the patients, aiming to promote and maintain the good health state of each person individually. In case of PA, the available services are the following [9]:

- Nutrition service: it is designated for all people that need to treat and prevent illness related with nutritional habits, who want to accomplish a desirable weight and increase his welfare but, must important, that want a health improvement especially in case of diabetics, obese, hypertension and other patients. It is a re-educational and nutritional counseling service that is made by a nutritionist every 15 days. The first appointment is designated to make a first evaluation, to elaborate a food plan and to define objectives. The next ones aim to accompany, keep the patient motivated and readjust the nutritional plan if it is necessary.
- Dermopharmacy service: it's designated to all population in order to identify each one's skin type and the most adequate products to apply to it. It is performed by the previously mentioned pharmacist that is specialized in dermocosmetic together with other specialist that goes to the pharmacy every month. Some diagnose tests and some personalized dermocosmetic counseling are done, according with patient's specific skin characteristics.
- Podiatry service: it is designated to patients that have mycoses, ingrown toenails, calluses, physiognomic feet and toes changes, walking problems and others, in order to improve patients feet health. It is done by a podiatrist who not only treats all the situations referred but also tries to prevent its appearance and re-occurrence.
- Diabetic's foot service: done by a specialized nurse, this service is offered to diabetic type I or II patients. It helps them to avoid feet wounds and, consequently, amputations, not only through patient counseling about this illness but also by making feet evaluation, teach patients the most adequate way of cut their toenails and treat their feet. It also allows to diagnose another pathologies and, if it is needed, to refer the patient to a specialized doctor.
- Chronic respiratory disease intervention: it is available to people that suffer from asthma, COPD or that present themselves in the pharmacy with respiratory complaints. The aim is to control these specific pathologies and improve patient's life quality, always doing a respiratory function evaluation, monitoring and control and, if applicable, identify some of these pathologies or if there are some medication related problems. It is done by a pharmacist through specific questionnaires application like GOLD (global initiative for chronic obstructive lung disease), mMRC

(modified medical research counselling dyspnea scale) and CARAT (control of allergic Rhinitis and asthma test) and also, if the patient fits in some special criteria, through spirometry tests and evaluation of FVC (forced vital capacity), FEV1 (forced expiratory volume in one second) and FEV1/FVC [10].

- Smoke cessation consultation: it is offered to all smokers, aiming to help them give up smoking, always strengthening the health benefits that one brings with it. To each patient, the pharmacist designs a personalized accompanying plan after his smoke habits evaluation.
- Pharmaceutical consultation: this one is one of the most embracing service because it is designed to all patients with uncontrolled diseases, polypharmacy, recurring therapeutic changes, multiple prescribers, patients with 65 years old or older or those with problems and doubts about their medication management. So, the pharmacist's role is helping them to control their chronic diseases, prevent medication duplication, interactions and adverse reactions and also alert them and their physicians if there are some changes or uncontrolled health problems, in order to improve drug's efficacy and patient's life quality. For that, in the first appointment it is required that the patient brings his "medication bag" and his last analysis results so that the pharmacist, after the first patient interview, can make a complete study and evaluation. To do this pharmacotherapy follow-up, it is applied a simplified form of Dader's Method. This service is the one that highlights the pharmacist's role as the drug specialist and most distinguishes him from the other health professionals present in the pharmacy. I had the opportunity to actively participate in one case integrated in this consultation. The patient went to the pharmacy and, after some talk with her, she referred concerns about dizziness, cold sweats and tremors. After some evaluation, we concluded that she was a diabetic person and that her diabetes was not controlled. We have intervened aiming to know her eating and routine habits, her medication dosages and how she administrated it. She referred that she spent long periods without eating and confessed that she was not taking Metformin according with the prescribed dosage because she felt that that drug was too strong. We instructed her to register the glycaemia values daily and, after a week, to return to the pharmacy. During that week, the pharmacist responsible for the service and I, had done a study about her prescription characteristics and, when the patient returned, we analyzed her glycaemia and concluded that it was ok so, something was wrong in the patient health history. One of the hypotheses was that the patient was misrepresenting the values. The other one was that she was measure fasting glycaemia after taking Pioglitazone and Metformin. Unfortunately, my internship ended before the next appointment with the patient.
- Individualized medication preparation: also performed by a pharmacist, it is designed to all patients that have some difficulties in their medication management, especially those who take four or more different drugs and those who have troubles in

medication adherence. In a specific schedule, all drugs are packed in a totally sealed disposable packaging, called pillpack (Annex V) that allows the administration individualization for a whole week, always ensuring drugs safety, stability and efficacy. Each patient's daily medication is divided according with dosage and moment of administration (e.g. fasting, breakfast, lunch, tea, dinner, before sleep) always in a way that is understandable for a specific patient taking into account his individually educational level. Those drugs that have a special dosage regimen (e.g. Varfine) are also divided according with the doctor's guidelines, so it is of great importance that the pharmacist keeps a constant and good relationship, not only with the patients, but also with their physicians. In my internship, I passed two entire days preparing several patients individualized medication and, with it, understanding this service importance and how is mandatory to be concentrated in this task performance, allowing to avoid mistakes that could put in risk patients health and disease control.

- Vaccines and injectable drugs administration: this service is done by a specialized pharmacist and can be applied to all the vaccines that are not contemplated in the national vaccination plan, aiming to reduce the morbidity and mortality related with infectious diseases like the flu, hepatitis, uterus cancer and others.
- Audition rehabilitation service: it is designed to all patients with audition problems who want to correct and improve their hearing. It is performed by a specialist in this area that recurs to video checking.
- Health-check: done with pharmacists and pharmacy technicians' help, it is designed to all people that want to monitor and control their health through biochemical and other parameters measure like BMI, fat mass, weight, height, blood pressure, capillary blood glucose, cholesterol (total, LDL and HDL), triglycerides, uric acid, pregnancy test, hemoglobin, HbA1C, PSA, and others. Related with these biochemical parameters, in the pharmacy it is kept a file that contains all important definitions, healthy target values to each parameter, non-pharmacologic measures and advices that can be given to the patients for their disease control and, most important, the procedures to perform in order to all biochemical tests be done correctly. Except BMI, weight, height and fat mass determination, all the other tests are done through enzyme-subtract reactions that, after the resulting products are formed, are quantitatively measured by spectrophotometry that, at the end, will correspond to a specific value of the parameter that were measured. I have learned how to perform all these tests and had the opportunity of doing some of them to patients that required it or to those that we counseled, after we realized that the values could be out of the acceptable margins. I remember a situation of a man with a very high rosuvastatin dosage prescribed that, after we asked if his cholesterol was controlled, he confessed that his last total cholesterol values were around 300mg/dL but, nonetheless, he continued to eat pork meet and sauces in big dinners with his friends

every week. So, we invited him to do the total-cholesterol test (since a total dyslipidemia test required that patients be in fasting) and, after we had obtained a value of 260mg/dL, we alerted him for the risk of a non-controlled cholesterol value, mainly because atherosclerosis development, and reinforced the importance of doing exercise and a diet with low-fat meet and vegetables, always avoiding red meet, sauces, stuffing meet and other unhealthy foods.

In the pharmacy, once a patient individual evaluation is performed, it can be necessary to recommend more than one of these services. For example, a diabetic patient who goes to a podiatry appointment can be forwarded to the nutrition area, to a diabetic's foot appointment and also to the pharmaceutical following and individualized medication preparation, in case that the diabetes or another concomitant pathology isn't controlled or if the patient has some trouble with his drug management.

During my internship, I had the opportunity to contact with almost all the services referred. However, the ones that I have participated more were the Individualized Medication Preparation, the Nutrition Service, not only by addressing some patients about it and, in some cases, also being able of convince them to make an appointment, but also because I had the opportunity of having a free appointment with the nutritionist for myself where I have also realized the way that she works with patients, the Podiatry Service, since that I had the chance of assisting to two different appointments and see what and how things are done according with each patient type, the Dermocosmetic Area through some case studies given to me by our specialized pharmacist and by assisting to various appointments with patients, and the Health-check service, where more than once, I measured blood pressure and performed some biochemical tests.

2.2.7 Prescriptions' Analysis and Checking

Nowadays, the majority of prescriptions are computerized and the prescribed drugs are referred by their INM (international nonproprietary name). The aim of this is to emphasis patients' choice, since there are a lot of generic and brand laboratories available with different commercial prices for the same API and dosage, which are grouped in the same CNPEM (código nacional de prescrição eletrónica de medicamentos). To better understand it, each code organizes drugs according with mandatory criteria which are: equal INM, dosage, dosage form and units number. This also allows the identification of cheaper drugs for the patients and simplifies the prescription checking done by pharmacists and pharmacy technicians [11].

After a few weeks of my internship, I was taught how to check all types of medical prescriptions. Then, that was one of my tasks in the pharmacy, always doing it with supervision or double checking by another pharmacist. It is important to refer that all prescriptions, with no exception, are triple checked by three different pharmacy staff members: first of all, during drugs dispense, the one that is attending the patient needs to be

focused in all important prescription points (see below) and make sure that everything is within legal parameters. Then, another staff element does the first check and, hereafter, a different element performs the second one. In the end, all prescriptions are verified one last time, organized according with their subsystem and batch numbers in groups of 30 prescriptions and each record group is emitted by the pharmacy's DT.

The important points that must be verified in all prescriptions are [5]:

- Three essential code bars which correspond to prescription number (on the top), doctor identification (on the left) and prescription place identification (on the right);
- Patient data: name and his SNS (health national system) number card, health subsystem number (if applicable), special contribution regimens ("R" for retired patients and "O" for patients who have some other special contribution regimen identified by a specific diploma);
- Drug Identification: as I said, normally each drug is identified by its API along with the correspondent dosage, pharmaceutical form, package dimension and drug homogeneous groups identification code bar. If the drugs are prescribed through its brand name, besides the previous described information, the prescription must also include drugs commercial name and the register drug number code bar [(CNP – product national code)];
- In special contributions regimens, beside the "O" reference, the correspondent dispatch must also be present;
- There can only be prescribed at most four different drugs, in a total of four packages in each prescription. For the same drug, the maximum number of packages is two. The only exception is for unitary dosage drugs in which can be prescribed in four equal packages;
- The prescription date must always be checked because it cannot be dispensed any drug of a prescription that is already expired. There are two prescription types: the normal one, that is available during 30 days after following the prescription emission, and the renewable one in which each prescription via is valid for a period of 6 months;
- The doctors signature must always be present in the end of the prescription;
- In the back of the prescription it is printed all the vital information in order to the government/health subsystem can pay the pharmacy participation towards medicine costs. This information includes patients' subsystems, the pharmacy identification, the correspondent prescription identification numbers and the dispensed drugs, the dispense date, along with the dispenser signature and pharmacy stamp that must be present and readable. It must always be checked if the dispensed drug fits with the prescribed one or if it belongs to the same homogeneous group. For that, a drug code printed in the back of the prescription must be included in the drug group that appears after the prescribed drug bar code is read. Note that, in some cases,

particularly if exception c) is evoked, if the dispensed drug of that particular homogeneous group is a generic, its value must be inferior of the prescribed drug value.

In the case of manual prescriptions that, at the present, rarely appear, the prescriber, the prescription place and the patient contribution subsystem type must always be identified but, in this case, this is done through visa stickers. In case of the prescription is allocated to a retired patient, the prescription place visa must be green. If the prescriber works in a private practice, the place can also be identified by a stamp or manual inscription, however that is not mandatory. It is required that the respective legal exception that justifies why a manual prescription has been prescribed must be signaled. The only valid reasons are: computer failure, prescriber's unsuitability, patient' home prescription or if there are only prescribed 40 or less prescriptions a month. Manual prescriptions are only valid for 30 days so it cannot be prescribed a renewable one of this kind. They can also not have any erasure and the calligraphy type must be uniform and equal in the whole prescription [5].

Sometimes drug prescriptions are made specifically through their brand or holder name. However, these situations must only occur if a specific drug does not have a similar one or if does not exist a similar reimbursed correspondent generic. So, the pharmacist needs to dispense the specific brand drug prescribed. The other situations that address the prescription by brand name are the presence of one of the following exceptions [5]:

- Exception a): medication with low therapeutic index contained in "Deliberação N.º 70/CD/2012", being the list composed by only three APIs: cyclosporine, levothyroxine and tacrolimus;
- Exception b): Previous adverse drug reaction;
- Exception c): treatment continuity for more than 28 days. With this one, the patient can chose a similar drug but only if its price is lower than the price of the prescribed drug.

In what drug prices are concerned, the staff must be warned that every three months some drug prices are altered and their duty is to update them as early as possible. The deadlines are automatically updated in the informatic system, however there is establish a flow period to sell products with previous prices. In both products reception and selling, it is always necessary to confirm if the written boxes' prices are coherent with the updated prices, being this task much easier now that the electronic prescription emits a red alert if the prices are out of date.

In the last day of each month, all the records resumes and correspondent prescription groups, along with billing documentation, are sent to ANF and/or health regional administration, depending of the subsystem prescription batch. There, all prescriptions are strictly analyzed and, if all points are within the legal boundaries, all the money that corresponds to each prescription contribution (depending of each patient subsystem) is refunded to the pharmacy.

2.2.8 Attending and patients' care

Over the last years, the traditional pharmacy concept has been changing, since the patient pharmaceutical approach is no longer just to dispense medicines but, mostly and most important, trying to establish a trustable relationship with them and to create a health space for each one's individual needs. For that, in PA is applied the Holon concept, which consists not only in dispensing the drugs required by a patient, but always to understand what are his health concerns, counseling him according to his specific problems, presented or deducted through prescriptions and their health complains analysis, and offer the best service possible. This aims to serve patients in the best way and, with it, provide them welfare and promote their health. Meanwhile, it is always very important that the patients feel confidence and trust in our work [12].

During my internship, I have learned that, for this concept to work and to have an excellent patient attending, all professionals need to apply their best attitude, personal and interpersonal behavioral techniques but, beside it, it is also of great importance for the entire staff to have and to apply their scientific knowledge, always adapting the language to patient's type and characteristics. According with the Pharmaceutical Deontological Code [2] and, especially to maintain all professionals actualized about all new information related with drugs and the pharmaceutical areas in general, the pharmacy has the obligation of giving staff training in diverse and new pharmaceutical areas, as it has happened a lot during my three months in the pharmacy, as I previously said. It is also important to keep the staff updated about all the new or adapted rules each time that INFARMED launches a new informative circular. Following, there are some examples of those important regulations that got out during my internship and that we have had access:

- List of 20 generic drugs that got out of the market after EMA has detected some bioequivalence trials unconformities in an Indian enterprise;
- List of non-prescription drugs exclusively dispensed in pharmacies (MNSRM-DEF), which is a group of drugs that previously were subject to a medical prescription but now, can only be dispensed in pharmacies and not in parapharmacies. Note that those drugs dispense must be according with their authorized therapeutic indications and recommendations [13]. Some examples are: Ibuprofen 400mg, oral Theophylline, topical Hydrocortisone, oral Tamsulosin, oral Pancreatine and others;
- Revision conclusion about Ambroxol and Bromexina that alerts for possible serious cutaneous allergic reactions like multiform erythema and Steven-Johnson syndrome [14];
- Beginning of the revision about codeine in children, since this molecule can be ultra-fast metabolized in morphine and, consequently, increasing the plasma morphine concentration which leads to toxic effects like respiratory difficulties [15];

- Ibuprofen and Dexibuprofen revision safety conclusion that confirmed the increasing of cardiovascular problems risk in patients taking doses greater than 2400mg/day [16].

In order to help all the staff members to do a better pharmaceutical counseling and approach to patients that reveal a specific health problem when they go to the pharmacy, the Holon Group have created some counseling protocols that help both pharmacists and pharmacy technicians to know how to resolute that problem. Of course that there are any protocols for all the diseases and patients' concerns, however, those that already exist are directed for the most common cases that appear in a pharmacy. So, to be more specific, there are counseling protocols for: acne, alopecia, skin changes in pregnancy, insomnia and anxiety, heartburn, vesicles, headache (e.g. in Annex VI), nasal congestion, flu, emergency contraception, elderly malnutrition, diarrhea, musculoskeletal pains, fever, sore throat, hemorrhoids, lip herpes, feet hydration, obesity, constipation, xeroftalmia, earache, athlete's foot, pediculosis, tired legs, insect bites, solar burns, rhinorrhea, cough, red eyes, eye's bacterial infections, onychomycosis and psoriasis. The general common points to all of them are their structure and the schematic way in which they are presented. Of course that in it all is included not only pharmacological measures but also non-pharmacological ones. There are also some cross-selling products that will help the patient to get better and prevent new events of the same problem, and also some medical and other services forwarding criteria.

In order to adequate the pharmaceutical service to each patient's needs, Holon Group has also created three standardized attending levels. At the same time that I started to watch and help in some patients' attending, I was also taught how to approach each person according with these levels characteristics. So, to summarize, Level 1 is practiced by all pharmacy staff members in the public zone or in one of the service counters and is designed to all pharmacy's patients. It is focused on the non-prescription drugs dispense and it is the first step to guide them to another attending level, if it is needed. Level 2, exclusively performed by pharmacists, is oriented for patients that have some illnesses that need to be monitored and controlled by specialized pharmaceutical interventions. That can be done in the sitting service counter or appeal to the pharmaceutical consultation, analyzing the most adequate biochemical parameters related with patient pathologies, if it is applicable. This level is the path to refer the patient for Level 3, being this one always done in a private room. It is appropriate to patients that need pharmaceutical evaluation or some specialized service in the pharmacy like nutrition, podiatric consultation or others [12].

In PA, the professionals are not limited to dispense a medical prescription or a drug required by a patient without any justification. So, in order to make a complete attending, and depending of a previous patient behavior analysis made by the professional who's attending him, we always try to comprehend what are the bases patient's pathologies through a simple prescription review and by doing open questions that will allow us to understand the event that justified the pharmacy recurrence. For example, if a patient requires a non-prescribed drug or presents a prescription written in an emergency acute context, first of all we always

try to understand what happened and then give the appropriate pharmacologic and non-pharmacologic counsels, according to the situation, the patient's request and his behavior, and the prescribed product. For example, a woman went to the pharmacy complaining about vaginal itch and warmth. Since it was a lady that was already in menopause, before we dispense any product, we asked about other health problems and concomitant administrated drugs that could be the reason for the event. However, after the gather all the information, we have concluded that it could be a primary sign of infection accompanied with dehydration, so we give her an appropriate cleaning gel, a hydration vaginal ointment and also Roter Cystiberry®, in order to treat and prevent the infection symptoms and signs.

If the patients present themselves with a prescription of usual drugs designated for their chronic diseases, we approach them in order to understand if their health state is controlled, if there are some adverse reactions or interactions related with medication and if there is something else that preoccupies them. If that is the case, and after making a situation study during the attending, we try to resolve those problems by counseling pharmacologic and non-pharmacologic measures, through patients forwarding for appropriate pharmacy services or even reference to a medical consultation, if justified. I remember a situation of an elderly man that, besides other chronic medication, was taking amlodipine to control his hypertension and angina pectoris. He complained about ankles and extremities swelling that had started about a month before. Since amlodipine is associated with that specific adverse reaction, we explained him that probably the edema was related with that drug and we counseled him to go to his doctor to explain him the situation and, probably after it, to adequate dosage and therapeutic in order to minimize that event. Besides it, nowadays there are more and more people that seek pharmacists not only to buy health products, but also to clear doubts about health care, health problems and medication. I had the opportunity not only to assist to some of these situations but, most important, to actively participate in some. For example, once I answered the phone and a very preoccupied lady asked if Acutil interfered with her heart disease and hypertension medication and so I explained her that there were no interferences, I also explained that Acutil is only a vitamin B12 and omega-3 supplement that helps the brain function and improves memory, especially in older people. Another time, a young girl went to the pharmacy concerned about the fact that she could be pregnant because she had sexual relations in the same day that she has forgotten to take her contraceptive pill. So, first of all, I calmed her down and explained that in single cases that she forgets to take it, in case of only have passed 12 or fewer hours, she can take the forgotten and the daily pill in the same day with reduced risk of getting pregnant. However, I reinforced the idea that if it already have been passed more than 12h after the forgetfulness, she can take the both pills as soon as possible but, depending of the cycle week, she needed to use additional contraceptive measures.

It cannot also be forgotten that, during the attending, all staff members fill a personalized label that contains each drug posology in a simple and understandable way. Besides it, there

are also present in it the pharmacy name, the patient name, in case that he has a monitoring informatics form in Sifarma, and some other advertences and information related with each medication in order for patients be alert for the way that must take their pills and some cares to take when do it.

The Holon Group also promotes some community health promotion projects targeted for some chronic patients like diabetics and patients with hypertension, in order to help them maintain their health state and encourage good and healthy daily habits. Over my internship period, we applied two of these projects, named "Colon-rectal Cancer Screening" and "Do you know if you're sleeping well?", being this last one based on questionnaires applied to all patients, but specifically for those with sleep complains or that asked for drugs in order to help them sleep better. Then, we sent the collected data to the pharmacy group headquarter in order to be performed a national study impact. In what Cancer Screening is concerned, the aim was to screen occult blood in faeces. It was destined to patients between 50 and 74 years-old that did not do a colonoscopy in the last 5 years, since the colon-rectal cancer is a very frequent cancer in Portuguese population and, as soon as people start to prevent it and as soon as it is detected, the better is the therapeutic response and success healing rate will be. I think that I had a big role in this project since I was responsible for preparing all the analysis kits which patients took (three analysis cups aiming to store faeces analysis of three consecutive days and a protocol with special instructions that patients must follow), the record sheets and patients' cards [17]. I also needed to explain to some patients the importance of this screening, how and when they must collect samples and when they must bring them to the pharmacy. The day that all patients delivered their samples, I also was the one who packaged and labeled them in order to go well identified and packaged to the analysis laboratory in Lisbon. It is relevant to say that we had three patients with positive results and, for them, the responsible pharmacist has done a recommendation letter for each patient's doctor and contact them to talk about the results.

2.3 Conclusion

In all my internship period, I had the opportunity to follow and actively participate in all tasks that involve drugs, pharmaceutical products, services and, most important, patients. I have started only in the orders area but, with time passing, I evolved to other areas like services participation, patients attending, drugs dispense, pharmaceutical counseling and accompaniment, and biochemical parameters measure and interpretation. I also had the opportunity of learning some points about good pharmacy management performance and how to overcome the barriers that actual Portuguese economic situation has put in the pharmacy area. However, one of the most important points for me in these three months was the way that all staff welcomed and accepted me, making of me an equal team and family member. They all supported and helped me when I had doubts or had done some mistakes, teaching me how to correct them and making of me a better future pharmacist. However, the most

important of it all was that they were capable of totally modifying my opinion about the community pharmacy area and making me fall in love with it. So, I can only be grateful to them all!

Chapter 3 – Hospital Pharmacy

3.1 Introduction

Hospital pharmacy services are the main responsible for ensuring all patients drug therapies, always with quality, safety and efficacy. They also belong to the hospital health care teams and participate in scientific investigation as well as in the educational area.

I have done my internship in the pharmaceutical services (PS) of "Hospitalar Center of Cova da Beira (CHCB), EPE" for 8 weeks, being these services accredited by the Joint Commission International (JCI), following the rule ISO 9001:2008. During this time, I had the opportunity to pass in all sections, having contact with all the work done there. These pharmacy principal areas are: logistics and storage, distribution circuits, pharmacotechnics, prescription validation, pharmacokinetics, pharmacovigilance, clinical trials and clinical pharmacy. Next are described all the important points of each section as well as some activities that was able to participate in these 2 months.

3.2 Pharmaceutical services organization and management – logistic and acquisition section

This PS section is the main responsible for the following activities: selection, acquisition, reception, storage and distribution of all drugs, pharmaceutical products and medical devices in the hospital. Only the individual daily distribution in single dosage (IDDSD), the ambulatory distribution and the drugs subject to special distribution circuits like the hemoderivatives, narcotic and psychotropic drugs are not of the responsibility of this section. So, this is the section that makes available all drugs and products referred above, always on time and in good conditions.

3.2.1 Drugs' selection

During selection, it is mandatory always to be present the pharmacist responsible for this section. He owes to be part of the therapeutic and pharmacy commission (CFT) in order to make a selection according to patients and hospital therapeutical needs. This selection must also be economically reliable for the institution. All the selected medication must be included in the national hospital medication formulary (NHMF) and in the CHCB pharmaceutical guide. The responsible pharmacist, through information drugs standardization, collects all data about each pharmaceutical product and, after it, discusses in CFT reunions opportunities to accumulate prescriptions in order to get better acquisition conditions. Aiming to update the hospital pharmaceutical guide, this pharmacist is also responsible for making economic

impact studies to drugs already included in this guide but for those that could be hereafter included if they show some advantages to patients. Exceptionally, if a doctor needs to prescribe a drug that is not included in this guide, he needs to fill in a specific form where it is included the therapeutic reasons that justify this guide specific drug introduction. After all the CFT members' analysis, the request is authorized or rejected. In case of drugs that need a previous exceptional authorization use (AUE), like those that do not have an approved AIM in Portugal but have been proved their clinical benefits especially in diseases without any other alternative, it is mandatory that the drug applicant fill in another specific import form which clinically justifies the authorization. This form is sent for INFARMED where it waits for approval or disapproval.

3.2.2 Drugs' acquisition

After pharmaceutical products selection, it is needed to acquire these products, being this responsibility shared with the hospital logistic (HL) services. Rationally speaking, before the acquisition, some consumption estimates are labored based on the average monthly consumption in the current year and comparing it with consumption of the same month of the previous year. So, a continuous update and evaluation of ordering points, maximal stocks considering an average consumption during 7 weeks, minimal stocks, amounts to acquire and other points must be performed. There must also be established a theoretical stock for urgent drugs, once there could be a massive consumption of these ones in case of some emergency. All drugs and medical devices have an ordering point considering the average consumption along 21 days. When a product reaches this point, the informatics system sends an alert to make its order. However, before it, the responsible pharmacist analyses the current consumption and stock as well as its consumption in the pass months. In order to evaluate a product real need, this pharmacist also studies and predicts the future consumption of it. In its request it is mandatory to specify if the product is urgent or not, since the urgent requests arrive in 48h, contrary to normal requests that take 7 days to arrive [1].

The acquisition can be done through centralize public application, using the "Serviços Partilhados do Ministério da Saúde" catalogue. The other acquisition options are: public application organized by CHCB; direct negotiation with laboratories and suppliers, direct purchase with the AIM holder; urgent purchase or loan application to local suppliers in case of products market break. In case of benzodiazepines, psychotropic and narcotic drugs, it is required a form filling (Annex VII of the Portuguese Coin House) [1].

In terms of quality indicators, in this section is necessary to monthly monitor the number of urgent requests performed and the number of market ruptures of pharmaceutical products.

3.2.3 Pharmaceutical products reception

After selection and acquisition, PS, together with HL services, must give entry to the ordered pharmaceutical products. This is done in a specific place with exterior access allowing charges and discharges. In the reception, there must always be present a therapeutic and diagnose technician (TDT) as well as a HL employee. All these procedures make sure that pharmaceutical products reception is supervised and updated. Conformity of new products It is also checked.

Initially, the refrigerated drugs are immediately stored in cooling chambers to ensure their stability. Then, quantitative and qualitative verifications are done using a delivery note issued in duplicated by HL services and signed for a TDT. The original form is filed in PS and the duplicate is filed in HL services. In this note there is information about batches and expiring dates that, during the reception and in order to verify whether they fulfill the conformity criteria or not, are compared with the same information on the package. If some nonconformity is present, one of the products is new or its supplier has changed, it is mandatory to alert the responsible sector pharmacist. It is important to refer that hemoderivates and raw materials must be accompanied with an analysis report and, in some cases, with its security form. If that do not happen, those products stay on quarantine until the forms reception. Cytotoxic drugs reception is done apart from other products, always verifying these products conformity and integrity. If a cytotoxic leakage happens there, a special kit placed in the reception space must be used. Talking about narcotic and psychotropic drugs reception, it is needed to always be present a pharmacist that, besides all the parameters talked above, also verifies if all packages are sealed. After the reception of all pharmaceutical products, they are stored in the central storage (store 10) or in specific places designed to store disinfectants, large volume injectable products and flammable products.

In this section, the main quality indicators goal is the monitoring of drug slaughter rate. The quality indicator that measure this goal is the monitoring of the nonconformity detected in drugs and pharmaceutical products reception.

3.2.4 Storage

The majority of pharmaceutical products are stored in store 10, being this one articulated with store 20 (ambulatory distribution section), store 13 (pharmacotechnics sector), store 11 (Fundão's hospital), store 12 (IDSSD section), store 18 (quarantine section) and with semi-automatic distribution systems like Pixys™. The storage is done by an operational assistant (OA) under supervision of a TDT, except in narcotic and psychotropic drugs that are stored directly by a TDT. For all products their storage must be done according with the rule "First-In-First-Out", meaning that products with a lower expiring date must be put in front of the others aiming to be consumed before the them. There are some products that must be

properly labeled before being distributed. Their label must contain the INM, dosage, expiring date and batch number, and all the products that suffer this process must be registered and quantified [2].

In store 10, products are placed according with their INM alphabetical order [2]. In the general section there are placed products of frequent use, dressing material, antibiotics, tuberculostatic drugs, eye drops, products for the ambulatory section, pediatric milks, anticonception products and products used in stomatology. There is also an area to store cytotoxic drugs, in which shelving are inverted and flagged to avoid products leakages and falls, another to enteric and parental nutrition store, a double lock safe where are put all narcotic and psychotropic drugs, and cooling chambers for refrigerated products. All raw materials are stored in the pharmacotechnics lab. Larger volume injectable products and disinfectants are placed in separated rooms. All flammable products are in a room with specific conditions (outwards fire door, internal walls that can contain fire, adequate electric system and others) that allows the quickly control of an accident.

In order to minimize risks and mistakes in patient's medication, CHCB PS have created a unique signage that alert for same drug's different dosages. These systems are: colors codes (red for the biggest dosage, yellow for the intermediate one and green for the lowest dosage); identical drugs names (STOP signal), potentially dangerous medication (yellow triangle) and electrolytes that need obligatory dilution.

To ensure this service quality, it is mandatory to do a stock and an expiring date's internal control in all storages. In both 10 and 12 stores, daily counts are done taking into account the pharmaceutical products ABC classification, in which A and B products are more frequently counted than C classified products. Enteric and parental nutrition, narcotic and psychotropic drugs, refrigerated products and other are counted in specific scheduled days. The counting values are then confronted with the informatics platform values and, in case of some divergence, there must be performed a data exchange between stores in order to understand the error's origin and normalize it. Related with expiring dates control, every month are done qualitative audits to signal all the products whose expiring date will end in 4 months and which can be flown to other services that have a higher consumption rate. This is one of this sector's quality indicators, along with the monitoring of regulations done in store 10.

3.2.5 Distribution

One of the most important activities in any hospital PS is the adequate and timely drugs and other pharmaceutical products distribution, aiming to accomplish the best health and life quality possible for each patient. For each patient, every supplied medication needs to be individually and informatically registered, aiming to monitoring their pharmaceutical therapy. This procedure is integrated in the information and management PS system of CHCB [3].

The CHCB PS are responsible for different types of pharmaceutical products distribution: traditional distribution, replacement levels distribution, Pyxis™ semi-automatic distribution, IDSSD and ambulatory distribution.

3.2.5.1 Traditional distribution

In each hospital service, there are predefined drugs and pharmaceutical products stocks acquired through PS traditional distribution. All the stocks reposition is done by TDTs and OA after it has been received an electronic requisition made by the head nurse of each requiring service. If the request is done until 2p.m., products are sent in the same day, otherwise they are only sent in the next day. When que request is received, a list of all asked products is issued to facilitate not only their dispense but also their following checking. In order to all products stocks be correct, before being sent to the respective services, it is mandatory to electronically impute them. When they arrive to a service, a nurse needs to check their conformity. Every three months, stock counts are done in the different hospital services aiming to normalize any inconformity and perform products consumption studies.

3.2.5.2 Stocks replacement levels distribution

Taking into account some services specific characteristics and ensure continuous pharmaceutical products accessibility, these ones need to have restrict and adapted stock periodical reset. This stock has a controlled and defined composition being controlled by the responsible pharmacist together with the service director and head nurse. This kind of distribution is done using "cars" or a semi-automatic distribution system called Pyxis™.

The services visits done by TDTs are monitored, since it is a sector's quality objective to accomplish. Its quality indicators are the monitoring of the distribution complains number and the monitoring of the number of interventions done to control all PS stocks.

Stocks replacement levels distribution through "cars" loading and exchange

In these "cars" there are individualized drawers with a fixed composition, in which their reposition is periodically done by the responsible OA recurring to an optical reader (PDA – personal digital assistant). This device automatically imputes all the consumptions done in each reposition. However, reposition is only done after it has been analyzed what drugs are missing in each "car" compartment.

The CHCB services that have this kind of distribution are: intensive care unit (ICU), stokes unit, neonatology, obstetric emergency service, medical emergency vehicle and ambulatory surgery unit. "Cars" verification and reposition are rotationally done and, in the end of each month, besides products dispense, expiring dates are also controlled.

Pyxis™ semi-automatic distribution system

In this system, there are minimal and maximum defined stock levels for each pharmaceutical product. When any product is taken off the system, a consumption register is generated in a way that, when the minimal stock of that product is reached it will be in the reposition products list. To access to Pyxis system and perform products replacement, it is necessary a fingerprint identification. Then, all the pharmaceutical products that are going to be replaced are selected in order to, drawer to drawer, the replaced units number and their expiring dates be registered in this system, always confirming the products stocks and respective expiring dates. Every month there are products collections whose expiring dates are almost at the end, aiming to increase patient's safety and minimize drugs inherent risks. For psychotropic and narcotic drugs, their reposition is done weekly by a pharmacist responsible for it. In CHCB there are four Pyxis™ systems: surgery room, general and pediatric emergency services and continuing and acute care unit (UCAD).

3.3 Ambulatory distribution

A lot of patients are subject to pharmaceutical therapies exclusively dispensed in hospitals. This aims to reduce the hospitalization costs, the nosocomial infections risks, as well as making possible for patients to continue their therapies in a familiar environment in case of they do not need special hospital cares. So, ambulatory distribution was created aiming to increase patient's life quality and their respective pharmaceutical treatments efficacy. This kind of distribution also allows a better control and pharmacovigilance of some therapies, once all patients' therapeutic compliance and drugs' adverse effects are monitored. This control and surveillance are needed due to some specific pathologies characteristics, drugs possible toxic effects and their huge economic value. All ambulatory dispensed drugs are free for patients, being this another advantage of this sector. So, in order to all safety and quality objectives be fulfilled, the pharmacist is the only PS professional responsible for this area, being always supported by an informatics program to facilitate all the procedures and registers [4-5]. This program also gives indication about the dispensed drugs, patients and their diagnosis information, adverse reactions and therapies costs. In there it is also registered patients names, their process numbers, their beneficiary numbers and financial entity names, address and phone numbers, all hospital appointments and respective episodes numbers and dates, the prescriber doctor, the pharmacist responsible for drugs dispense, their costs and dispense dates, the dispense legal authorization, the ambulatory pharmacotherapeutic history and finally some relevant observations. It is also important to have appropriate facilities so patients can have some privacy and, at the same time, allowing good drugs storage and preservation conditions.

Ambulatory dispense is made for patients allocated to the external hospital appointments section, daily hospital patients, after hospitalization discharge and, in some cases, after an emergency visit. Every dispensed drug in ambulatory regimen aim to treat the following legislated pathologies: oncologic diseases, psychiatric diseases, chronic renal failure, transplantation, HIV/SIDA, multiple sclerosis, lateral amyotrophic sclerosis, hepatitis C, cystic fibrosis, Lennox-Gastaut syndrome, Machado Joseph disease, acromegaly, hemophilia, paramyloidosis, familiar planning, growth hormone disease, tuberculosis, rheumatoid arthritis and Allagille-Fallot syndrome. There is also the possibility the ambulatory drug dispense for non-legislated pathologies like pulmonary hypertension, hepatitis B, serious osteoporosis, hepatic and intestinal transplanted patients treated with new immunosuppressive drugs, and others [6].

In terms of ambulatory dispense, first of all the prescription must be received by the responsible pharmacist. Except for specific legislated situations, prescriptions are always informatically done. It contains patients' identification and the respective beneficiary number, the prescriber doctor's name, the emission date, drugs INM, posology, dosage, pharmaceutical presentation and the quantity to dispense or the predicted therapeutic time. Next, the pharmacist proceeds to the prescription validation always verifying dosage, quantity, previous therapies and if the prescribed medication is approved by CFT. In case of any doubt or problem, the pharmacist made contact with the doctor in order to resolve any question, also giving some relevant information if it is needed or requested. After it, the pharmacist prepares the dispensed drug, always informing patients about how it should be administrated, its posology and possible adverse reactions occurrence and also encourage patients to adhere to the pharmacologic therapeutic in order to accomplish the desire effects. The medication is then dispensed to patients, accompanied with oral and written information, specific informative papers elaborated by PS pharmacists and, if applicable, some pictograms showing the time and how to take medication. Finally, it is necessary to make the dispense imputation in the informatics system, not only to correct products stocks but also to register all dispensed medication for each patient. For that, the pharmacist must include in each process the INM drug, dosage, pharmaceutical presentation, dispensed quantity and drugs batches. The batches are extremely important since all medication in the ambulatory section is imputed through its batch number. It is also necessary to identify the prescriber doctor and the correspondent prescription episode number. In the end, the system provides an imputation number that must be registered. In cases of paper prescriptions, it must be signed by the pharmacist and the person that presented the prescription and, in the end, transcribed to the informatics system. In every dispense cases, patients must be incentive to adhere and make a responsible use of the medication, being alerted to report any adverse reaction felt to a doctor or a pharmacist. If it is the first time that a patient takes medication in the CHCB ambulatory section, he must sign a responsibility term [6-7].

According with "Despacho nº 18419/2010" of PL, CHCB PS are allowed to dispense medication for patients of other public or private health institutions that suffer of rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, polyarticular juvenile idiopathic arthritis and plaque psoriasis. So, biological drugs that are prescribed according with this dispatch can be prescribed by public entities or private practice offices correctly certified by "Direcção Geral de Saúde". In these cases, pay billings are sent for the respective regional health administration of the preceptor entity [8].

In the majority of cases, the ambulatory section can only dispense medication for a month period, even if the therapeutic time is higher. Prescriptions must be renewed in each appointment and medication is periodically taken in a divided way. Dispense can be made directly to the patient or to a caregiver, always recording the dispense date and the identification numbers of the person who did it. However, there are two exceptions, one being related with contraceptive pills that can be given for a period of 3 months, and the other one is the medication mail shipping for 2 months. This last case can only be applied to patients who live at more than 25km of the hospital. This medication needs to have a monetary value of less than 50 euros and cannot be refrigerated products, hemoderivates, thalidomide and contraceptives.

In this sector it is required to perform all patients monitoring, taking special attention to drug interactions, allergic reactions and therapeutic adherence. If any non-adherence case is detected, the pharmacist must alert the prescriber doctor through phone or by an appropriate form. Pharmacovigilance is also done in the ambulatory section not only to guarantee that all product stocks are enough to fulfil patient's needs but also to evaluate the adherence, mainly in cases of multiple sclerosis, hepatitis B and C, pulmonary hypertension, biologic drugs therapies, lateral amyotrophic sclerosis and other pathologies. It is important to say that every day it is performed verification of the previous day prescriptions, always confirming the patient name, dispensed drugs and its respective dosage, dispensed quantity and batch number, imputation number and the respective cost center. In case of non-conformities, they are always corrected.

In the ambulatory section, stock counting is weekly done, except for the narcotic and psychotropic drugs. This sector is also responsible for hemoderivate, narcotic and psychotropic drugs circuits. These products existences, batch numbers and expiring dates need to be controlled by a responsible pharmacist in every hospital services.

In this sector there are quality control objectives and indicators. Those are following described: increasing available flyers numbers to be given to patients, monitoring the correct imputation to cost centers and decrease the regularization number in all stocks counting.

In my internship I have stayed in this section for 2 weeks. There, I had the opportunity of accompanying the majority of dispenses, collaborate in daily prescriptions verification, do weekly stock counts and make the ambulatory pharmaceutical products orders reception. I

was also present in some patient active pharmacovigilance, mainly those with multiple sclerosis taking fampridine or with hepatitis C taking sofosbuvir+ledispavir. To do this pharmacovigilance, patients must be questioned about possible drugs related adverse reactions and concomitant therapies, aiming to understand if there are some interactions between therapies. I have also performed some medication dispenses, always with a pharmacist next to me. Besides, I have elaborated a flyer related with dexamethasone pills and I have transcribed some others for the new PS layout. I have also done dugs identifying labels to better organize the archived flyers.

3.3.1 Special controlled drugs' distribution

CHCB PS established that the distribution of plasma derived drugs (hemoderivates), narcotic and psychotropic drugs is one of the ambulatory sector responsibilities, making their distribution to all hospital services and, punctually, for patients in ambulatory regimen (ex: hemophilic patients).

3.3.1.1 Hemoderivates

There are a lot of risks related with this drug group, being the most important the blood infectious diseases transmission. So, it is mandatory to have a strict control and traceability in order to establish a cause-effect relationship between drug administrations and the occurrence of a disease. According with "*Despacho nº 1015/2000, 14 de Setembro*" of PL, registers of these drugs clinical requisition, distribution and administration must be done [9]. For that there is a special form – Annex I of the Portuguese Coin House – containing a "pharmacy via", signed and filled in the ambulatory section, and a "service via" taken and filled. Each form contains patient's and doctor's identification (Table A), as well as the prescribed drugs names and the prescription justification (Table B). Along the prescription validation, if the pharmacist has any doubt he contacts the responsible doctor. Each supplied unit is properly identified with labels containing the patient name and process number, the clinic episode, the requesting service and other relevant data. In Table C the responsible pharmacist registers the individual drug batch number and the INFARMED release certification of the respective batch, the supplier laboratory and the quantity dispensed. In the end, forms need to be signed and dated by the pharmacist who made the dispensation and by the requesting service employee. Finally, each dispensed hemoderivate need to be informatically imputed and the respective imputation number must be registered in the "pharmacy via" before being filled. If the medication is not administrated to a patient in a 24h period and if it was maintained in its specific conservation conditions, this one must be returned to PS. In those cases, the nurse must register the devolution in Table D of the "service via" as well as the respective devolution date and signature. At the return moment, the pharmacist needs to informatically register the devolution, being its number and quantity noted in the "pharmacy via" [10].

Every month, hemoderivates circuits are randomly closed, mostly in those hospital services that present non-conformities in Table D filling by nurses. When the pharmacist goes to each service, he confers the "service via" form, confirming the administration date, the administrated hemoderivate and respective dosage and quantity, its batch number and the supplier lab, the nurse signature and identification number. In case of devolutions, the pharmacist must also verify if all is correctly filled.

In my internship, I actively participated in hemoderivates dispense as well as in the Annex I filling and drugs informatics imputation. I have also the opportunity of visit the obstetric, surgery 1 and medicine services in order to close the hemoderivates circuit, always verifying if all the parameters are correct, signaling and correcting those ones which are not.

3.3.1.2 Narcotic and psychotropic drugs

Ambulatory sector is also responsible for the narcotic and psychotropic distribution circuit. All services need to have a specific form called "Annex X of the Portuguese coin house". In each individual service requisition, only one active substance can be asked, however, this one can be requested for more than one patient, since they are properly identified with their name and process numbers. It must also be identified the dosage, the administrated quantity and the administration date of each drug [11].

In every service and according with their needs, there is a predefined stock, being these ones placed in a double lock safe. In every service request, annex X must be present and properly filled by nurses and signed by the service director. After the pharmacist validates all annex X information and proceed to drugs dispense, both service employee and pharmacist must sign and date the requisition. The original form is filed in the ambulatory section and the duplicate is taken for the requesting service. The informatics imputation is done recurring to each drug batch number and, in the end, an imputation number is registered in the original form. In the next day, all requisitions must be verified and sent to the secretary, in order to generate a consumption map of the narcotic and psychotropic drugs used in medical treatments and their total movements performed in PS. This map is sent to INFARMED every three months [11].

Instead of have a double lock safe, in some clinical services narcotic and psychotropic drugs are stores in the Pyxis™ system. Weekly, their reposition is done only by an allocated ambulatory pharmacist accompanied with a Pyxis™ list containing the patient's names who consumed this medication, the consumed quantity and the responsible nurse name and identification number. In services that have narcotic and psychotropic drugs, every month the ambulatory pharmacist goes there to count them, also verifying their expiring dates and batches numbers, informing the responsible nurse in order to correct any existent non-conformities. In my internship I had the opportunity to follow this control in services like medicine 1, surgery 1, pediatric service, UCAD, stoke unit and medical specialties service, following all records and corrections performed. I also have the opportunity to follow all the

narcotic and psychotropic procedures as well as help dispensing and impute them, aiming to understand their circuit and process.

3.4 Individual daily distribution in single dosage (IDDSD)

Nowadays, every drugs have some associated risks being most of the times reveled by the presence of adverse effects. Besides, with the population aging and the increase of polimedicated patients, drugs interactions are an increasing concern. So, it is essential to develop strategies and mechanisms to increase drugs safety, not only when a drug is prescribed and administrated to a patient, but also to cover all the drug circuit. It is also imperative to rationalize all therapeutics, reducing their costs and wastes [4-5]. For these reasons, IDDSD is one of the most important areas regarding all hospital pharmacy functions and objectives. Besides, and since hospital pharmacists' functions in drug management, therapeutic revisions and pharmacotherapeutic profiles creation are increasing, the IDDSD allows a greater dedication to these functions, once the pharmacist is the drugs specialist. In a short cut, the IDDSD functions are: drugs repacking, medical prescriptions interpretation and validation, pharmacotherapeutic profiles creation and, after it, drugs distribution. In this section the responsible professionals are all pharmacists.

This kind of distribution is mainly intended to hospitalized patients, beginning after an electronic medical prescription reception [4-5]. Since CHCB has more than one different informatics system, it is essential having an interface between the medical support system (SAM) and the drugs circuit integrated management system (SGICM), enabling pharmacists to reception and validate all medical prescriptions. This informatics communication is valid to all clinical services except for ICU and stokes unit, once both have a different informatics system that do not make connection with SGICM. In these cases, the prescriptions are presented in a table that needs to be transcribed to PS informatics system and, at the same time, making their validation. A pharmacotherapeutic map is then generated for each hospitalized patient in both services. After the tables transcriptions are done, these ones are eliminated from the shared folder in order to decrease errors risk [12].

To be validated, every medical prescription must contain the following information: patient name, birth date, process number, hospitalization service, doctor's name, INM of the active substances prescribed, pharmaceutical presentation, dose, dosage and the prescription date. For antibiotics, it is mandatory to establish a therapy end date with renewal possibility, if necessary. For the pharmacist, this allows a better control of antibiotics resistant bacteria, a therapeutic rationalization and also patient's clinical evolution monitoring and possible iatrogenic reactions related with antibiotics.

Electronic prescriptions bought some advantages like the minimization of errors in patient's medication. Over time, it also allows professionals to optimize the system by the creation of a data base with alerts and information about some drugs. This aims to help not only the prescriptions done by doctors but also their validation and pharmaceutical interventions done by pharmacists. These alerts are mainly related with possible drug interactions, maximal dosages, antibacterial therapeutic time, individualize adverse reactions and other important information related with drugs. The electronic prescription also allows the registration of all the pharmaceutical therapeutic profile of each patient along his hospitalization. There is also the possibility of filling a form intending to justify a specific antibiotic administration according with the drug policy implemented by CFT.

After each service prescriptions daily reception (orthopedic, medicine 1 and 2, gastroenterology, surgery 1 and 2, obstetrics, gynecology, psychiatry, pediatric, medical specialties, surgical specialties, ICU, UCAD, stokes unit and pulmonology), each pharmacist validate them, being an extremely important step since it is in here that are detected possible drug interactions, drugs duplication and possible adverse or hypersensitivity reactions to a drug. There is also studied the prescribed antibiotics, mainly those with a restrict use (e.g. amikacin, linezolid), and if each patient pharmacotherapy is according with CHCB pharmacotherapeutic guide. During the validation step, there are also selected those drugs dispensed through unitary dose distribution and the other ones that, at the first time are distributed by this way but after it are distributed through the traditional ways. In this last case, it is included all the multidose products (e.g. insulins, larger volume injectable products, inhalators, oral solutions, ointments), estimating their consumption calculating the administrated dose and their administration time. This aims to avoid wastes or administration errors and, in the end, rationalizing all therapeutic. In case of the pharmacist detects any prescription inconformity or has any doubt, he must immediately contact the responsible doctor and register this intervention in the PS interventional register system.

After the validation is done, the pharmacotherapeutic map is generated and sent to the responsible TDTs of this section. Medication for a period of 24h must be prepared, with weekend's exception when is necessary to prepare medication for a period of 48h and 72h. In CHCB PS, the medication preparation is done recurring to semi-automatic systems: Kardex e fast dispensing system (FDS). Both aim to reduce errors numbers and the preparation time, increasing the executed work quality and rationalizing products' stocks. FDS is directly linked with SGISM, aiming to repack solid pharmaceutical oral products (pills and capsules) with high turnover. For both equipment, there is a strict control of each drug batch number and expiring date. CHCB PS also create unique signs, aiming to avoid possible distribution exchanges and errors. For example, for different and partial dosages there is a color system, being the red for highest dosage, yellow for intermediate dosage and green for the lowest one. For similar medication names the implemented sign system is called LASA (Look Alike, Sound Alike), highlighting the different syllables in order to draw attention for this question

(e.g. CeFRAdine and CeFTRAZidime). For patients with similar names hospitalized in the same service, it is mandatory that the label has the "identical names" information printed, minimizing the administration errors. There are other signs like products identification that need "obligatory dilution", being these considered high risk medications once their administration without dilution can fatal to the patient. Some injectable products also have the inscription of "partial dosage".

In this section, medication is prepared in modules designed for different services, being these ones divided by drawers which are ordered by beds' numbers. The identification label is identified with the patient name, birth date, process number, hospital service, bed number and dispensing date. Each drawer is divided in 4 compartments (morning, evening, night/SOS) with exception of the psychiatry service drawers (morning, lunch, evening, supper/SOS). The remaining medication (larger volume products, enteric or parenteral nutrition and others) are identified with a similar drawer label and are transported in identified boxes with the service name and the dispensing data. The refrigerated products are maintained in cooling chambers until their distribution time, having a special label with the information "keep in the fridge" [12].

Pharmacists also perform a qualitative and quantitative verification of all modules. In the 2 weeks of my internship in this section, I had an active role in this verification. After the consumptions have been imputed by a pharmacist, OA proceed to medication distribution in a pre-defined schedule. The first medication delivering is done at 2p.m. and the last one, destined to medicine 1 and 2 services, are done at 4:30p.m. Until each service delivery time, the pharmacist systematically verify if there are any alterations to the initial prescription, discharges or new hospitalized patients, making the modules update according with those changes. Over the day, even after 7p.m., some urgent requests can appear. For those ones the response must be quick and delivered by an OA of the PS at pre-determined times. In case of existing drugs that have not been administrated to patients, they have to be returned to PS in the same way that has been distributed. All devolutions must the counted and informatically reverted by the TCTs, generating a list of all reverted products organized by service and devolution date [12].

In this section, besides the antibiotic therapy strict control, mainly for those that are of exclusive hospital use, there is a list of drugs subject to batch traceability. This list contains hematopoiesis stimulating factors, immunomodulators and antineoplastic drugs, anti-infectious drugs (antivirals), monoclonal antibodies and others. Toxic effects and adverse reactions are inherent to this kind of drugs once they are recent and have few available security information. So, to know which batch is associated to each patient, aiming to relation a batch number with a possible adverse reaction, all appropriate required registers for these drugs are made.

The IDDSD quality objective is monitoring the distributed medication errors numbers. In order to fulfil this objective, the non-conformity percentage must be less than 1%. The remaining quality indicators of this section are: monitoring the regularization number performed in store 12, monitoring the storage non-conformities number in store 12 and monitoring the schedule compliance in delivering medication to each service.

Besides having accompanied the prescription validation, verifying the drawers medication and make some perfusion dosage calculation, I also have an active role in the conformities or non-conformities registration. I have also been continuously stimulated to do some researches about the patient's prescribed medication, mainly related with unusual pathologies or off-label drugs uses (e.g. Rifaximine is used in cases of hepatic encephalopathy however this use is not officially described in Portugal). I have also done some research about dose adjustments according with each patient weight body superficial area (e.g. IV Ondansetron used in a hospitalized child probably in a higher dose that the one allowed) and about antibiotic's adverse reactions and interactions.

3.5 Pharmacotechnics

Aiming to prepare safe and effective pharmaceutical products, considering that sometimes the pharmaceutical industry cannot supply all the requested therapeutics, CHCB PS have developed processes and procedures to accomplish these objectives, recurring to adequate means, structures, material and human recourses, always ensuring a quality system in pharmaceutical formulations preparation [4-5].

The pharmacotechnics sector is divided in 5 areas: sterile injectable cytotoxic and biologic medication preparation; parenteral nutrition and other sterile solutions preparation; non-sterile compound medicines preparation; repackaging; purified water preparation.

3.5.1 Sterile injectable cytotoxic and biologic medication preparation

Cytotoxic drugs belong to a pharmaceutical group with toxicity and staff contamination inherent risks. Since these are expensive therapies that require time and care in their preparation, the CHCB has centralized their production in PS in order to decrease costs and preparation errors.

During the 2 weeks of my internship in this area, I have realized that there are different protocols designed to each specific cancer treatment, most of them consisting of pre-medication, for chemotherapy adverse effects treatment, and with more than one cytotoxic drug. In a way to better understand and relate these therapies with different diagnosis, I have done a survey of some patients protocols (Annex VII). The most common chemotherapy's

adverse effects are nausea and vomiting. In order to treat them it is used, in monotherapy or in association, Ondansetron, dexamethasone, metoclopramide, domperidone or prednisolone defined schemes. Diarrhea can also be a common adverse effect, mainly in protocols containing irinotecan. So, due to its parasympathycolitics effects and being and acetylcholine antagonist, atropine is used in pre-medication regimen to treat this adverse effect. If some hypersensitivity reactions occur, clemastin is the chosen drug once it is a histaminergic antagonist. Less frequently drugs like paracetamol, ranitidine, hydroxyzine, some electrolytes and other are used to treat these effects.

Cytotoxic preparation begins after the daily hospital nurse makes the prescription confirmation, after evaluating if the patient can or cannot receive the treatment. Then, the prescription is informatically sent, being this one validated by one of the sector pharmacists. In this validation it must be taken into account if the prescribed doses are well adapted to patient's weight, body superficial area, high, age, sex, and creatinine clearence. If there are some problems with a prescription, the responsible doctor must be contacted and, after it, it must be done dosage adjustments and justifications. After has been validated, a therapeutical map for each patient is printed in duplicate (one of the copies in filed in PH and the other one is taken along with the medication to each service). In this map the patient's data (name, age, weight, high, body surface area and creatinine), the requesting hospital service, diagnosis, protocols name, number and cycle day and what medication should be administrated must be present. For each drug a label is also printed. In order to fulfil all quality JCI requests, these labels must contain patient's name and process number, bed number, drug INM, total dosage and volume to administrate after reconstitution/dilution of each drug, perfusion debit (mL/h) and duration, administration date, stability and conservation cares as well as the highlighted reference to "Cytotoxic". In many cases, cytotoxic drugs must be diluted or reconstituted in NaCl 0.9% or in glucose 5%, being necessary that all used products be informatically imputed through their batch number and expiring date aiming to trace and control all products stocks. Next, all pre-medication, raw material and equipment are prepared and put in the preparation chamber transfer, always reviewing the therapeutic map [13].

Cytotoxic and biologic drugs are prepared in clean chambers with vertical laminar flow in a way that not only the preparation is protected against contaminations but also the operator and the surrounding environment. In order to maintain the aseptic conditions of the chamber air, there are some HEPA filters and a continuous air flow that allows the required pressure maintenance. All days, both chamber pressure and temperature are controlled in order to never exceed the acceptability criteria to make cytotoxic or biologic preparations (T<25ºC, preparation room pressure<0mmH20 and antechamber pressure>1mmH2O). It is in the antechamber that the operator performs hands cleaning and disinfection, equipping itself with a sleeve and frontal enhanced coat, aiming to avoid damages in case of spills, a cap, feet special protections, gloves and a P3 mask that protects the operator from inhaling

cytotoxic aerosols and vapors. In order to increase the manipulation safety, *luer-lock* connections are used in syringes and in other perfusion equipment, as well as spikes to avoid aerosols production. In the end of all produced cytotoxic recurring to NaCl or glucose, those are always wrapped in aluminum paper aiming to cover the original reconstitution/dilution solution label, once the parameters in there do not correspond to the recipient constitution after the cytotoxic preparation. So, a new label is put there to identify not only the contained drug but also if it is a "Vesicant", "Irritating" or only a "Cytotoxic" drug. After the preparation, all the used material is properly discarded for specific contents and then taken to be incinerated. Then, the cytotoxic drugs, the pre-medication and the cytotoxic duplicate form are put in special bags in order to an OA proceed to their transport to the requiring service, always in hermetic containers identified with "Cytotoxic Transport" label. In the service it is required a nurse's signature and the reception hour registration, in order to posteriorly count the difference between the reception and the prescription confirmation time. This time is one of the quality indicators of this section, being defined that the waiting time in less than 3% of all patients cannot pass the 2 hours. The other quality indicators of this area are: monitoring the air quality in vertical air flow chamber, monitoring the microbiologic control of the vertical chamber surface and monitoring the microbiologic control of the sterile products prepared in the vertical chamber [13].

During the 2 weeks of my internship in this area, I have observed the cytotoxic preparation and prepared the pre-medication to some patients. I have also the opportunity to prepare a Rituximab solution, since it is a monoclonal antibody with low toxic effects or inherent contamination risks. I have also filled and create some therapeutic profiles, made some perceptions confirmation through phone always noting the confirmation time aiming that, at the end of the day, be able to calculate the time waiting average of all patients of that day. I have also accompanied the weekly patients' surveys who are receiving chemotherapy, in order to make the following week's planning. I have also done some product's counting, and accompanying the protocols validation, verifying if all dosages and correspondent cycles are correct. I have also the opportunity to collaborate in updating some products security files, contacting each product responsible enterprise thought telephone or email to request that information.

3.5.2 Parenteral nutrition and other sterile solutions preparation

In this area it is mainly prepared parenteral nutrition bags designed to patients that, due to their specific pathologies characteristics, cannot be fed through the enteric way. This type of nutrition is also applicable to severe cases of malnutrition, in highly countersunk patients or for those ones in the pre-surgical period. In here, some eye drops are prepared, being Bevacizumab one of the examples most prepared in CHCB PS, aiming to treat macular eye edema though intravitreal administration.

Every day it is necessary to make all prescriptions reception, always confirming if there are some surplus nutrition bags in any infirmary. This request is made because of the highest monetary value of each parenteral nutrition bag and, once they are kept in the fridge, they have an expiring date of 7 days and 48h after have been taken out the fridge. So, their reuse can be equated even for the same patient or to another one that has a similar NP prescription. After this procedure, the prescription is validated and the dispense date is registered in the informatics system, as well as the prescribed protocol (parenteral nutrition bag type and additives), bags batches and serial numbers, additives and waters used in NP reconstitution batches, the doctor's name, the perfusion rhythm (mL/h) and data about quality control. Next, label and the preparation form are impressed. In the label there must always be the patient's name and process number, bed and service identifications, if it is a central or peripheral parenteral nutrition bag, its components, doctor's name, perfusion rhythm, expiring date, the preparation date, the operator signature and the time of the perfusion beginning. The peripheral bags are destined to be prefunded through minor caliber veins, being this the reason why their osmolality (800mmOs) is lower, once the central parenteral nutrition bags are designed to be administrated in central veins with a much higher caliber. All of them are tricompartmentalized (lipids, glycose and aminoacids) in order to maintain their safety and stability until the reconstitution time. After all these procedures and reunion of the necessary raw material and equipment, those are put in the horizontal flow chamber transfer. This chamber has some characteristics that allow products protection from external contaminators. It is in the antechamber that the operator put his equipment (sterile coat, gloves, feet protection, mask and cap), making the hands washing and disinfection in order to proceed to the manipulate preparation. For the parenteral nutrition bags preparation, first of all it is done a glycose and aminoacids mixing and homogenization step, followed by the water-soluble oligoelements addiction and homogenization and, only after it, the lipids are mixed and homogenized. Then the lipo- and water-soluble multivitamins are reconstituted with 5mL of water in order to be injected and homogenized inside the parenteral nutrition bag, preventing any precipitation formation. According with the type and branch bad, these steps could differ. After preparation, all used material is discharged and the chamber is cleaned. Compound medicines must be properly conditioned according with their characteristics, validated (in case of rejection it must be created a justification form which is attached to the preparation form) and labeled [13].

In this area there are also some quality indicators being them the monitoring of the active air in horizontal airflow chamber, the monitoring of the surface horizontal chamber microbiologic control and the monitoring of the sterile product prepared in this chamber.

Since the first day, I was able to perform the bags lifting in different services, preparing some peripheral 1275mL and central 1275mL and 1870mL parenteral nutrition bags, only doped with multivitamins and oligoelements. I have also prepared two samples (5mL of glucose 5%+5mL of water to injections) for microbiologic control in the horizontal chamber.

3.5.3 Non-sterile compound medicines preparation

There are some patients that specifically need products that are not produced by pharmaceutical industry, being necessary for PS to prepare them, always after a service requisition (in specific scheduled days, unless there are some emergency) or after a medical prescription. The most common pharmaceutical forms prepared are: ointments, oral solutions and suspensions for pediatric use, solutions aimed to be to be topically of for diagnosis use ad others. It is important to refer that this non-sterile compound medicines need to be approved by INFARMED, being also multidose preparations or distributed in multiple packages for unitary administration. These products are prepared in an independent laboratory of the one used to prepared sterile compound medicines, cytotoxic and biological drugs. Here, there are two types of material: internal use material, designed to prepare products that aims to be internally administrated, and external use material, to prepared extemporaneous products. In this lab, there are some raw materials that always need to be accompanied with a valid analysis report that must be filed in PS. In case of that report is not valid, products are maintained in quarantine until a valid report reception. Raw materials are stored taking into account their compatibility in identified places, with labels and, in some cases, with dangerous or toxicity grades signs [13].

Before the production beginning, it is mandatory to have a guide based on the requisition/prescription, selecting the required manipulate to proceed for the production guide emission containing information about the requiring service and, in some cases, the patient identification name and process number, the doctor's identification, the quantity that needs to be prepared, the batch number and supplier lab of the conditioning material. Next, is printed a preparation form and label containing the PS and the service director identification, the pharmaceutical presentation, INM, dosage, composition, quantity, administration route, posology, preparation date, the expiring date, conservation conditions, batch number, special care conditions, the requiring service and, if applicable, a label saying "External Use" with a red background.

The operator must equip himself and then gather all the material and equipment necessary, always verifying the material cleaning and safety state. In case of using volatile substances, it is very important to work inside a *hotte*, aiming to protect the operator. At the end, it is mandatory to measure the pH value of all internal preparations, making their register and comparing them with the tabled values or those contained in the historic register preparation for the same product. Finally, the labeling and the final conformity verification is done.

Here, one of the quality indicators is the compound medicines quality control, aiming that less than 1,5% do not fulfil the required criteria. Another indicator is the monitoring of the reception's non-conformities and validation errors in products designed only for pharmacotechnics.

In my internship, I have accompanied all the procedure described until now and, besides it, I also have the opportunity to prepare some compound medicines, always with supervision. I have done a nystatin suspension to treat all mouth problems related with chemotherapy and other pathologies, a cinchocaine+nitroglycerin ointment to teat anal fistulas, propranolol syrup and prednisolone syrup for pediatric use, a 10% formaldehyde solution and a acetic acid 3% solution for external use, a betamethasone ointment to treat a patient with psoriasis, and I have collaborated in the dosage adaptation of a tuberculostatics regimen consisted of rifampicin, isoniazid, ethambutol and pyrazinamide.

3.5.4 Repacking

As already told, CHCB PS are responsible for solid oral pharmaceutical forms (pills and capsules) repacking, aiming to be distributed in the ambulatory or in IDDSD sections. This task is of the TDTs responsibility, being always supervised by a pharmacist in order to ensure drugs quality and safety. To all entire pills and capsules that are not cytotoxic, photosensitive or thermolabile, a semi-automatic repackage system called FDS is used. However, first of all drugs must be taken off their original blister and then be charged in cassettes placed in specific positions that are confirmed through optical reading. In the informatics FDS program it is introduced information about medication batch numbers, expiring dates and preconized repacking quantities. Then, this system automatically calculates the expiring date after drugs repacking, being always of 6 months [14].

In case of partial dosage drugs, initially drugs fractioning must be done following their placement in another semi-automatic repackage system called MSAR (repackage semi-automatic machine). This machine is also used in the repackage of fractioned and entire pills, cytotoxic capsules, thermolabile and photosensitive drugs [14].

After the repackage, validation procedures must be performed, verifying not only drugs quality and integrity but also labels information (INM, pharmaceutical presentation, dosage, supplier laboratory, batch number, drugs original and repackage expiring dates and quantity of repacked drugs). This information is then evaluated and compared with the correspondent information included in the original drug's package and, after the charging daily report print, non-conformities are analyzed, being this a quality indicator of this section. The other quality indicators are: the monitoring of non-conformities in the FDS sleeve, discrepancies monitoring in the charging FDS stocks and the monitoring of non-conformities in MSRA repackage drugs.

3.5.5 Purified water preparation

As already said, PS are responsible for non-sterile compound medicines preparation, even for external or internal use. For internal use, compound medicines are prepared using water for injectable preparations. For external application products it is used purified water. In CHCB, this water is prepared using a purificator system called Micromeg, Instant Purified Water,

ELGA. The withdrawal water can only be consumed in the same day in order to have a better microbiologic control. So, in all cases, it must be register the name of who did the water extraction, the time of it, the extracted volume, always verifying the water quality and the system battery.

3.6 Pharmacovigilance and clinical pharmacy

The clinical pharmacy concept allowed that the pharmacist focus became the patient, always given the best pharmaceutical care with the least possible risks. The clinical pharmacists must be integrated in multidisciplinary teams, directly accompanying patients in different hospital services and providing continuous support for doctors and nurses [4-5]. The activities performed in the clinical pharmacy area are: encourage the pharmacotherapeutic CHCB guide use, control the antibacterial therapy time and the use of restricted antibiotics, monitoring the use of biologic and antiviral drugs, accompanying the artificial patient's nutrition, integrate in clinical reunions and service's visits, monitoring the serum drug's levels, elaborate protocols and guidelines in order to provide information about drugs to other health professionals [15].

In clinical services visits, the pharmacist, together with doctors, nurses, social assistants, psychologists and nutritionist, is one of the crucial elements in there. The visits are effectively done at the "patient head" in surgery 1 and 2, medicine 1 and 2 and gastroenterology services. Given the specific patients and service characteristics, in the stoke service, instead of clinical visits, there are done reunions with all the above referred professionals. In both, the pharmacist follows all patients' clinical histories and their correspondent pharmacotherapy, making possible to make reasonable interventions if it is needed. These interventions aim to decrease possible adverse reactions, rationalize patients' therapeutics in terms of pharmacotherapy and pharmacoeconomy areas, making a therapeutic optimization and clarifying any doubt about drugs. In my 2 weeks of internship in the IDDSD area, I have assisted to all reunions and clinical services visits being present in some interventions done by the responsible service pharmacist [15].

In terms of pharmacovigilance, the aim is to detect, evaluate and register possible adverse reactions even if these one have already been described. So, this allows studying these reactions incidence, gravity and causality, correlating them with a specific administrated drug. In case of any suspect adverse reaction, any health professional or patient must spontaneously notify it to INFARMED. There is a specific form, online or in paper format, that must be filled if there is any adverse reaction suspicious, being necessary to collect all data necessary for the notification. In all situations, a notification copy must stay in PS in order to be sent to CFT. Besides this pharmacovigilance type, CHCB also has an active pharmacovigilance system to monitoring new drugs introduced in the institution, highly risk

medicines and those ones that need additional monitoring, being these last ones identified by an inverted black triangle (e.g. biologic drugs). In these cases, notifications are performed by the responsible pharmacist after talking with health professional allocated to different hospital services or directly with patients, being all information registered in a form specifically designed for the effect. Medications errors must also be detected and classified according with their origin (prescription, transcription, preparation/dispense or administration errors) and severity (from A to I), and then added to a data base in order to be analyzed in order to apply an appropriate risk management strategy. The notification must be done to Quality Office and Risk Committee to be subsequently analyzed and treated in the pharmacy and in CFT [15].

For both clinical pharmacy and pharmacovigilance, there are some quality indicators, aiming to monitoring all therapeutics and pharmacist's interconnection with other services. For that, the quality indicators are the monitoring of the number of services visits done without any previous organized clinical visit and the dugs number included in the active pharmacovigilance.

3.7 Medication information

All drugs responsibilities are assigned to pharmacists and, given the huge quantity of new and updated information daily launched by the drugs responsible entities, it is mandatory to exist a continuous clarification and counseling to all patients and health professionals about these issues, ensuring the rational use of all medication. In terms of passive information, being it the information given when a health professional or a patient ask a question related with drugs and therapies, PS developed a system where all these asked questions are registered along with the pharmacist answer and the references used that supply it. It is also registered the answering time and the pharmacist name who answered the doubt. Initially, the pharmacist that receives the question search in the data system if there is already an answer previously given to the same question. If there is not, and in order to answer by the most scientifically correct way, some research is done so that the provided answer will be the most adequate and correct. Related with active information, being this one all the information given by PS by its own initiative after some necessities, failures and interests in learning something new about drugs, CHCB PS frequently publish some information like oral and injectable drugs preparation books, informative newsletters and others. In my internship, I have the opportunity to participate in this last type of information, since I have worked in the updating of multidose products expiring dates after its opening. To do it, first of all I needed to update these products list according the pharmacy existent stocks, and then contact some supplier's enterprises to ask the required information.

3.8 Pharmacokinetics

The pharmacokinetics area aims to correctly proceed to drugs administration. For that, serum levels must be measured in order to individualize each therapeutic control. Through this, correct dosage preconization can be done without risk of under- or overdose, mainly for drugs with a narrow therapeutic index and variable kinetic behavior [4-5]. In CHCB PS pharmacokinetic studies are only performed for vancomycin and gentamicin. Before doing it, it is necessary a previous evaluation need and having monitoring possibilities. If all these conditions are gathered, the monitoring proposal is done by a doctor or a pharmacist, filling an appropriate form for the effect.

In my internship, I had the opportunity of following the monitoring of a patient receiving gentamicin IV, done with help of the informatics program Abbottbase PKS. Before the monitoring starts, it is mandatory to schedule a time for blood samples collection (valley, peak or intermediate) and, only after the collection and recurring to this informatics program, the best posology regimen is established. Information about patient's age, sex, weight, high and about the therapy begging date, the administration route, frequency and dosage, serum creatinine concentration and blood concentration/time values must be obtained and introduced in the program. Then, following a mathematical bayesian model and a bicompartimental pharmacokinetic model, valley and peak concentrations are estimated. It can also be simulated different posology regimens being posteriorly analyzed in order to optimize and individualize the therapeutical regimen, always taking into account the infection type, the biochemist parameters, clinical evolution and others [16].

This area objective is monitoring the proposal acceptance number, needing that this percentage be greater than 90% to fulfil it.

3.9 Clinical trials

Nowadays, in Portugal clinical trials are regulated by "Lei nº21/2014, 16 de abril". Each trial can only begin after previous INFARMED authorization an evaluation, as well as by the ethical commission to clinical investigation and by the data protection national commission. After their approval, the trial promotor must gather with the enrolled pharmacists in order to give all the documentation and information about all the procedures, mainly the experimental drugs reception, store, dispense and possible devolution [17].

According with the previous referred law, the responsible pharmacists in this area must participate in the beginning reunions, organizing all the intern and promotor supplied documentation, being them the responsible for experimental drugs reception, dispense and devolution to the promotor. All these drugs associated registers (batch numbers, quantities,

expiring dates, devolutions and disables) must be kept in the pharmacy. Pharmacists are also responsible for register and control of the temperature, not only when drugs are stored but also throughout their transportation. If any standardized parameter is exceeded, the promotor must be immediately alerted [17].

In experimental drugs reception, the responsible pharmacist needs to access, through a unique password, to a specific site supplied by the promotor. In this electronic address, it is checked if a predetermined shipment is available in PS to posteriorly be allocated to trial CHCB patients. This medication storage must be done in a specific locked closet with temperature control. The only exception is for experimental drugs that need to be refrigerated. These ones are placed in a fridge in the clinical trials room. Both experimental drugs and their documentation have strict access. Before dispensing, the prescription, done in a special form, must be validated by the responsible pharmacist. After dispensing, patients must be counseled about the correct drug use and informed about the clinical trial. Besides, it must be emphasized to patients that they need to return to PS all the leftover drugs (except for cytotoxic experimental drugs), since the pharmacist need to evaluate patients compliance. Note that these drugs cannot be taken off the controlled storage conditions even if their expiring dates have passed, since stability studies can be done if required. This can only occur after the promotor informs that those drugs have to be destroyed. It is important to say that each experimental drug has a unique code allocated to a specific patient identified by a number followed by his initials' name letters. Dispensing registers must be performed [18].

In a way to better manage and evaluate each clinical trial, PS created several documents: the clinical trial diary, where it is registered all the participant patients' occurrences; the clinical trial summary only containing crucial information like the principal investigator name, contacts, trials types and objectives; stock registers where is registered the reception and dispense dates as well as batch numbers and expiring dates. All these documents need to be in the pharmacy for a period of 15 years [18].

At this moment, the majority of trials are allocated to the cardiology area. There is also a diabetics' related trial and other in the hematology-chemotherapy are. Finally, the other trial perform in CHCB is in the imunohemotherapy service.

3.10 Conclusion

In my hospital pharmacy internship, I have the opportunity to observe and participate in all areas and activities of this service responsibility, applying some of the acquire knowledge in all 5 years of the pharmaceutical science course. I have passed 2 weeks in each PS area, which not only increase my understanding about this sector, but also contributed to my

professional formation as a pharmacist. I have also realized that hospital pharmacists have an important role in all clinical services organization, providing information and therapeutical counseling to all the others health professionals, in order to increase patients' life quality and rationalize costs and wastes.

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Annexes

Annex I - Example of part of photossensibility drug induced research work performed

Reações de fotossensibilidade.

O conceito de fotossensibilidade diz respeito às alterações cutâneas, de características clínicas variadas, localizadas essencialmente nas áreas expostas e nas quais a luz tem um papel fisiopatológico fundamental.

A interação ao a nível da pele, entre uma substancia foto-activa (cromóforo) e a luz de comprimento de onda adequado induz uma sequencia de alterações fotofísicas, fotoquímicas e fotobiológicas, globalmente designadas por reação de fotossensibilidade. A sua etiologia pode ser devida a irradiação de cromóforos exógenos (medicamentos ou outras substancias químicas) em contacto com a pele, quer por aplicação local quer por administração sistémica.

A fotossensibilidade clínica de causa exógena é induzida pela exposição concomitante ou sequencial a certos agentes químicos (fotossensibilizantes) e a radiação não ionizante. Entre os agentes causais contam-se medicamentos de utilização tópica ou sistémica e diversas outras substâncias tais como: cosméticos, conservantes alimentares, produtos industriais e produtos agrícolas. A introdução das sulfamidas como agentes anti-infecciosos foi de imediato seguida por descrições clínicas devido a reações de fotossensibilidade. Os primeiros sistemas de farmacovigilância (Committee on Safety of Medicines), verificaram que a dimetilclortetraciclina, a protriptilina e o ácido nalidíxico eram os principais agentes fotossensibilizantes. Atualmente, é muito extensa a lista de fármacos sistémicos aos quais já foram atribuídas reacção de fotossensibilidade. Para várias destas substâncias este efeito adverso é frequente (acido nalidixico, amiodarona, azapropazona, cloropromazina, piroxicam, protriptilina, psoralenos, retinóides especialmente a isotretinoína, sulfamidas principalmente o cotrimoxazol, tetraciclinas, especialmente a dimetilclortetraciclina, e tiazidas). No entanto, tendo em conta as publicações mais recentes, é possível prever que os anti-inflamatórios não esteróides, as tetraciclinas, os fármacos citotóxicos anti-neoplásicos e algumas das novas quinolonas têm potencialidades para serem os principais fármacos fotossensibilizantes. De referir que algumas doenças subjacentes como o lúpus eritematoso sistémico e a porfiria podem favorecer o aparecimento de reação de fotossensibilidade

Classes Terapêuticas

- Anti-histamínicos - reação relacionadas com administrações tópicas e sistémicas

- Cetirizina (Cetix, Zarec, Zyrtec)
- Desloratadina (Aerius, Azomyr, Clarus, Dasselta, Denovals, Deslix, Neoclarityn)
- Difenidramina (Benaderma, Benergina, Benylin, Caladryl, Drenoflux,)
- Dimenidrinato (Arlevert, Draminal, Enjomin, Viabom, Vomidrine)
- Hidroxizina (Atarax)
- Loratadina (Claritine, Claridon)
- Prometazina (Fenergan)

- Anti-infecciosos

- Fluoroquinolonas:

- Ciprofloxacina (Cetraflux, Ciplox, Ciproxina, Estecina, Giroflox, Nivoflox, Oftacilox, Quinox,
- Levofloxacina (Levoxa, Oftaquix, Seltrix, Travanic),
- Moxifloxacina(Avelox, Proflox, Vigamox),
- Norfloxacina(Chibroxol, Noroxin, Uroflox),
- Ofloxacina (Audret, Bioquil, Exocin, Floxedol, Oflocet, Ottoflox).

- Tetraciclinas:

- Doxiciclina (Actidox 100, Doxytrex, Efracea, Periostat, Vibramicina)
- Minociclina (Arestin, Ciprancin, Minocin, Minotre)
- Oxitetraciclina (Terricina)
- Tetraciclina (Ciclobiótico, Cloridrato de tetraciclina)

Annex II - Example of part of inhalators devices research work performed

Dispositivos médicos para Inalação

Estes dispositivos permitem administrar fármacos diretamente nas vias aéreas. A via inalatória é preferível para o tratamento de muitas doenças respiratórias uma vez que se requer menor quantidade de fármaco, maior rapidez de acção e menores efeitos secundários, principalmente a nível sistémico. No entanto, para garantir a sua eficácia, é necessária uma boa utilização de cada um dos dispositivos inalatórios daí que a educação, quer do doente quer do profissional de saúde, para o seu melhor uso possível seja essencial.

Um passo inicial obrigatório a realizar por todos os doentes que administrem corticosteróides inalatórios é, após o final da inalação, bochechar bem com água e nunca degluti-la. Isto é importante de modo a evitar efeitos adversos destes fármacos como a candidíase oral ou a rouquidão.

É importante adequar escolher o inalador de acordo com as características do doente, ou seja, é necessário distinguir inaladores lentos (ex: idosos e crianças) de inaladores rápidos e vigorosos (ex: adultos). Sempre que possível utilizar inaladores com a mesma técnica, quer em terapias de manutenção quer como SOS. É importante também rever periodicamente a técnica de inalação.

1) **Inaladores de Pó seco**

- Turbohaler

Fármacos Disponíveis para este dispositivo

- Agonistas Beta-2 de Curta Duração
- *Bricanyl* 500mcg (Terbutalina) – AstraZeneca Produtos Farmacêuticos, Lda.

- Corticosteróides
- Pulmicort 200/400mcg (Budesonida) – AstraZeneca Produtos Farmacêuticos, Lda.

- Agonistas Beta-2 de Longa Duração
- *Oxis* 4,5/9mcg (Formoterol) – AstraZeneca Produtos Farmacêuticos, Lda.

- Associação Corticosteróides + Agonistas Beta-2
- *Assieme 80/160+4,5mcg* (Budesonida+Formeterol) – AstraZeneca Produtos Farmacêuticos, Lda.
- *Assieme 320+9mcg* (Budesonida+Formeterol) – AstraZeneca Produtos Farmacêuticos,

Modo de utilização
Retirar a tampa com o inalador na vertical

Rodar a base colorida para a direita e depois para a esquerda até ouvir "click"

Expirar forçadamente, longe do inalador

Colocar o bocal entre os lábios com o inalador na horizontal. Inspirar rápido e profundamente, no entanto o medicamento não se irá sentir fluir

Retirar da boca e manter a apneia durante 10 segundos aproximadamente. Expirar lentamente e fechar o dispositivo. Limpar o bocal com um pano seco, semanalmente. Nunca deve ser usada água ou outro líquido na limpeza do dispositivo. Deve-se evitar o armazenamento ou contacto com a humidade.

Annex III - FGP protocol for 70% saturated alcoholic solution of boric acid

Solução Alcoólica de Ácido Bórico à Saturação
(FGP A.II.1)

Forma farmacêutica: solução

Data de preparação: _____

Número do lote: _____

Quantidade a preparar: _____

Matérias-primas	Nº do lote	Origem	Farma-copeia	Quantidade para 100 g	Quantidade calculada	Quantidade pesada	Rubrica do Operador e data	Rubrica do Supervisor e data
Ácido bórico				5,0 g				
Álcool a 70 % *(V/V)*				q.b.p. 100 ml				

Preparação

	Rubrica do operador
1. Verificar o estado de limpeza do material a utilizar.	
2. Colocar em proveta rolhada uma quantidade de álcool a 70 % (V/V) correspondente a de cerca de ¾ da quantidade total de solução a preparar.	
3. Pesar o ácido bórico, e adicionar, aos poucos, ao álcool a 70% *(V/V)*, agitando fortemente durante 20 segundos, após cada adição.	
4. Após adição de todo o ácido bórico, completar o volume com álcool a 70 % *(V/V)* e agitar durante 20 segundos.	
5. Deixar a proveta em repouso durante 1 hora, agitando-a, durante 20 segundos, de 15 em 15 minutos. Início: _____ Final: _____	
6. Filtrar a solução obtida em 5.	
7. Lavar o material utilizado.	
8. Secar o material.	

Rubrica do Director Técnico	Data

Annex IV - Legislated INFARMED mathematical equation for prescribed-drugs price calculation

$$PVP_{c/IVA} = (PVA + MgA + feeA + MgF + feeF + TaxaInf.) \times 1{,}06$$

Annex V - PillBox for Individualized Medication Preparation Service

Annex VI - Headache approach Holon Group Protocol

Questões a colocar ao utente para avaliação da situação

- Quando teve início?
- Que tipo de dor sente? (caracterização da dor)
- Tem mais algum sintoma? Náuseas? Febre?
- Já tomou alguma medida terapêutica? Qual? Resultou?
- Sofre de alguma doença crónica (hipertensão arterial, sinusite, rinite...)?
- É frequente ter cefaleias?
- Associa o início da cefaleia a algum traumatismo, à exposição a algum fator ambiental (fumo, frio, odores intensos...), alterações no estilo de vida ou hábitos alimentares?
- Dorme bem? Fuma? Bebe café?
- Tem medido a sua pressão arterial?
- Tem alguma outra doença que esteja a tratar ou encontra-se a fazer algum tipo de medicação?
- Está grávida ou a amamentar?

Situações que requerem encaminhamento para nível 2 de intervenção ou referenciação ao médico

- O doente tenha idade inferior a 2 anos ou esteja grávida ou a amamentar;
- O doente tenha dor severa, rigidez, vómitos e/ou febre;
- O doente tenha sofrido um traumatismo recente;
- O doente apresente outros sintomas, outras patologias associadas ou medicação crónica associada;
- O doente descreva uma situação recorrente, sem resposta a terapêutica já instituída;
- A sintomatologia esteja associada a perturbação do sono, fadiga ou febre;
- Subsista a percepção do profissional de que pela intervenção prevista: o problema não se atenuará; outras patologias associadas se possam agravar; se pode alterar negativamente a efetividade e/ou segurança da medicação atual.

TRATAMENTO

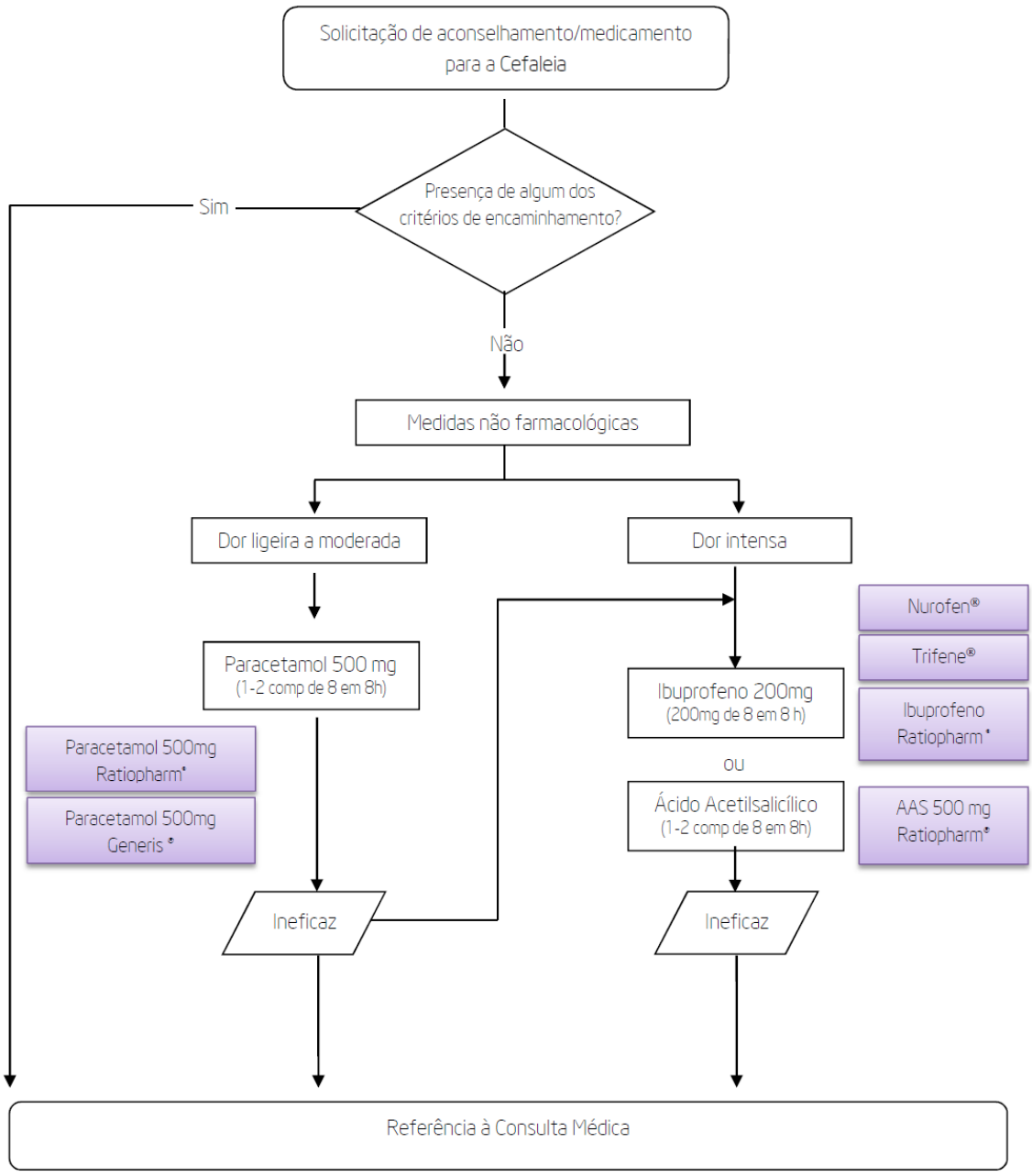
Farmacológico

- Analgésicos e anti-inflamatórios no alívio sintomático da dor;
- Os MNSRM devem ser tomados aos primeiros sinais ou sintomas;
- O ácido acetilsalicílico não deve ser recomendado a crianças com menos de 12 anos de idade devido à possibilidade de síndrome de Reye (doença rara, mas pode ser fatal; provoca encefalopatia e dano hepático);
- Não deve ser aconselhado qualquer anti-inflamatório a doentes com asma, rinite, prescrição de outros anti-inflamatórios não esteróides ou esteróides, prescrição de anticoagulantes orais, úlcera gástrica, hemorragias gastrointestinais, doenças inflamatórias crónicas do intestino ou gravidez;
- A terapêutica farmacológica deve ser instituída com precaução, devido ao risco de se mascarar patologia base mais grave.

Não Farmacológico

- Devem ser identificados os fatores predisponentes e tentar evitá-los;
- Exercício físico regular, boa hidratação e alimentação equilibrada;
- Deixar de fumar, evitar beber demasiado café e/ou bebidas com cafeína e/ou bebidas alcoólicas;
- Dormir o suficiente;
- Manutenção de uma postura corporal correta;
- Massagens nos músculos do pescoço, ombros ou cabeça, exercícios de relaxamento, tentar evitar o stress;
- Banho de água quente.

FLUXOGRAMA DE ACONSELHAMENTO



Annex VII – Chemotherapy patients' protocols

Diagnosis	Protocol	Pre-medication	Cytotoxic drugs	Reconstitution Solution	Dilution Solution
Non-Hodgkin Lymphoma	RCOP – 21 days	Paracetamol 1000mg Clemastine 2mg IV Ondansetron 8mg IV Ranitidine 300mg Prednisolone 20mg	Vincristine 1.4mg/m^2		NaCl 0.9% 100mL
			Cyclophosphamide 750mg/m^2	NaCl 0.9% 75mL	Glucose 50ug/mL 500mL
			Rituximab 375mg/m^2		NaCl 0.9% 500mL
Rheumatoid Arthritis			Methotrexate 25mg/mL		
Macular Edema (Ophthalmology)			Bevacizumab 2mg intravitreal injection		
Klatokim Tumor		Metoclopramide 10mg IV	Gemcitabine 1000mg/m^2		NaCl 0.9% 500mL
Breast Neoplasia	CMF breast	Dexamethasone 5mg IV Ondansetron 8mg IV	Methotrexate 40mg/m^2		NaCl 0.9% 100mL
			Fluorouracil 600mg/m^2		NaCl 0.9% 100mL
			Cyclophosphamide 600mg/m^2	NaCl 0.9% 50mL	NaCl 0.9% 100:250mL
Metastasized Rectum Neoplasia	5-FU+Isovorin+ Bevacizumab	Metoclopramide 10mg IV	Disodium Levofolinate 250mg/m^2		NaCl 0.9% 100:250mL
			Fluorouracil 500mg/m^2		NaCl 0.9% 100mL
			Bevacizumab 5 AUC		NaCl 0.9% 100mL

Colon Neoplasia	XELOX	Dexamethasone 10mg IV Ondansetron 8mg IV	Oxaliplatin 100mg/m^2		Glucose 50mg/mL 500mL
			Capecitabine 850mg/m^2		
Breast Neoplasia	Oral Vinorelbine		Vinorelbine 60mg/m^2		
Gastric Neoplasia	ECF	Dexamethasone 10mg IV Ondansetron 8mg IV Mannitol 20mg/mL	Fluorouracil 200mg/m^2		NaCl 0.9% 36.68mL
			Cisplatin 50mg/m^2		NaCl 0.9% 500mL
			Epirrubicin 50mg/m^2		NaCl 0.9% 100mL
			Potassium Chloride 74.5mg/mL + Magnesium Sulfate 200mg/mL + NaCl 0.9%		
Metastasized Colon Neoplasia	FOLFOX 6	Dexamethasone 10mg IV Ondansetron 8mg IV	Disodium Levofolinate 200mg/m^2		NaCl 0.9% 250mL
			Oxaliplatin 100mg/m^2		Glucose 50mg/mL 50mL
			Fluorouracil 400 + 2400mg/m^2		NaCl 0.9% 92mL
Metastasized Rectum Neoplasia	Cetuximab + Irinotecan	Clemastine 2mg IV Dexamethasone 10mg IV Ondansetron 8mg IV Atropine 0,25mg IV	Cetuximab 400 + 250mg/m^2		NaCl 0.9% 1000mL
			Irinotecan 180mg/m^2		Glucose 50mg/mL 500mL
Acute Lymphoblastic Leukemia			Methotrexate		

Lung Adenocarcinoma state IV	Gemcitabine/ Carboplatin	Dexamethasone 10mg IV Ondansetron 8mg IV	Gemcitabine 1250mg/m^2		NaCl 0.9% 250mL
			Carboplatin 5 AUC		Glucose 50mg/mL 100mL
Lung Adenocarcinoma	Oral Vinorelbine	Dexamethasone 4mg IV Ondansetron 8mg IV	Vinorelbine		
Klatskin Tumor	GEMOX	Metoclopramide 10mg IV Dexamethasone 10mg IV Ondansetron 8mg IV	Oxaplatin 100mg/m^2		Glucose 50mg/mL 500mL
			Gencitabine 1000mg/m^2		NaCl 0.9% 500mL
Primitive Lung Adenocarcinoma	Pemetraxede/Carboplatin	Dexamethasone 10mg IV Ondansetron 8mg IV	Pemetraxede 500mg/m^2	NaCl 0.9% 20mL	NaCl 0.9% 100:250mL
			Carboplatin 5 AUC		Glucose 50mg/mL 100mL
Multiple Myeloma	CYBORD	Dexamethasone 50mg	Bortezomib 1,3mg/m^2	NaCl 0.9% 50mL	
Malign Bladder Neoplasia	Imuno-BCG		Bacilo Calmette-Guerin 1U	NaCl 0.9% 50mL	NaCl 0.9% 50mL
Bladder uncertain Behavior Neoplasia	Mitomycine C		Mitomycine 40mg	NaCl 0.9% 40mL	NaCl 0.9% 40mL
Pompe Disease (Neurology)	Alglucosidase alfa		Alglucosidase alfa 20mg/kg	Water for injection preparation	NaCl 0.9%
Breast Neoplasia	Transtuzumab	Clemastine 2mg Ranitidine 50mg Dexamethasone 10 + 5mg IV	Transtuzumab 6+8mg/kg	Water for injection preparation	NaCl 0.9% 250mL

Metastasized Rectum Neoplasia	FOLFOX 6 Na	Dexamethasone 10mg IV Ondansetron 8mg IV	Fluorouracil 400 + 2400mg/m^2		NaCl 0.9%
			Oxaplatin 100mg/m^2		Glucose 50mg/mL 500mL
			Disodium Levofolinate 200mg/m^2		NaCl 0.9% 250mL
Rectum Neoplasia	Cetuximab/Irinotecan	Clemastine 2mg IV Dexamethasone 10mg IV Ondansetron 8mg IV Atropine 0,25mg IV	Irinotecan 180mg/m^2		Glucose 50mg/mL 500mL
			Cetuximab 400 + 250mg/m^2		NaCl 0.9% 1000mL
Metastasized Rectum Neoplasia	Cetuximab/FOLFIRI Na	Clemastine 2mg IV Dexamethasone 10mg IV Ondansetron 8mg IV Atropine 0,25mg IV	Fluorouracil 400 + 2400mg/m^2		NaCl 0.9%
			Irinotecan 100mg/m^2		NaCl 0.9% 500mL
			Disodium Levofolinate 200mg/m^2		NaCl 0.9% 250mL
			Cetuximab 400 + 250mg/m^2		NaCl 0.9% 1000mL
Gastric Neoplasia	Gastric Macdonald	Metoclopramide 10mg IV	Fluorouracil 425mg/m^2		
			Disodium Levofolinate 10mg/m^2		NaCl 0.9% 250mL
Ovarian Neoplasia	Paclitaxel/Carboplatin AUC 6	Ranitidine 50mg IV Cemastine 2g IV Dexamethasone 10mg IV Ondansetron 8mg IV	Carboplatin 6 AUC		NaCl 0.9% 500mL
			Paclitaxel 175mg/m^2		NaCl 0.9% 250mL